University of Alberta

Supplementing Children with Arachidonic acid and Docosahexaenoic acid improves Visual perception

by

Vanessa Wing-Sze Lien

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

in

Nutrition and Metabolism

Department of Agricultural, Food and Nutritional Science

Edmonton, Alberta

Spring 2005

0-494-08130-9

Library and Archives Canada

Published Heritage Branch

395 Wellington Street
Ottawa ON K1A 0N4
Canada

Bibliothèque et Archives Canada

Direction du Patrimoine de l'édition

395, rue Wellington
Ottawa ON K1A 0N4
Canada

Your file *Votre référence*
ISBN:
Our file *Notre reference*
ISBN:

NOTICE:
The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

AVIS:
L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protège cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

Canada

University of Alberta

Library Release Form

Name of Author:	Vanessa Wing-Sze Lien
Title of thesis:	Supplementing Children with Arachidonic acid and Docosahexaenoic acid improves Visual perception
Degree:	Master of Science
Year this Degree Granted:	2005

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves all other publication and other rights in association with the copyright in the thesis, and except as herein before provided, neither the thesis nor any substantial portion thereof may be printer or otherwise reproduced in any material form whatever without the author's prior written permission.

Signature

Abstract

The objectives of this study were to 1) to determine dietary intakes of arachidonic acid (AA) and docosahexaenoic acid (DHA) in healthy children; 2) to supplement children (who consumed low intakes of DHA), with a nutritionally complete dietary formula containing AA and DHA; 3) to evaluate AA and DHA fatty acid status in the blood and visual perception. Children, 4-7 years of age, living in central Alberta, Canada, who consumed low intakes of DHA were recruited in a controlled, double blind study and assigned randomly into two groups: the experimental group was provided with a formula containing AA (20-30 mg/day) and DHA (14-21 mg/day) (long chain polyunsaturated fatty acid group (LCP group)) and the control group was provided with the same formula without AA and DHA. There was a general trend toward a higher level of AA and DHA content (RBC phospholipid and plasma phospholipids) in LCP group compared to control group at 7 months. This study is the first to show that daily supplementation with AA and DHA for a period of 7 months improved visual perception in children who were previously identified to have low dietary intakes of DHA.

Acknowledgements

I would like to express my sincere thanks to my supervisor Dr. M.T. Clandinin, for providing me with the opportunity to work on this project. Thank you for your guidance and continuous support throughout this degree

Thank you to my committee members, Dr. CJ Field and Dr. IM MacDonald; they were integral to the completion of my degree.

Thank you Mich Wilke for always listening to me and providing me with great advice, and thanks to Nancy Evans who was there to assist me during both research projects. I would also like to thank Dr. Goh for his technical assistance and statistical help. Thank you for your patience and always taking the time to answer all my questions. Thank you to the all the members of the Dr. Clandinin lab group for all their help, and thank you to the support staff of the Department of Agricultural, Food and Nutritional Science. I would also like to thank Dr. JR Michelson and Marni Pearce for helping out with the neuropsychological assessments, and thanks to Brad Wakeman and Michelle Lang who performed all the visual function assessments.

I would like express thanks to my parents, Herbert and Diana Lien for their love and support. Thank you to my best friend Iva Seto; she was there with me from the start to finish and for the good times and the bad. Thanks to both my family and friends for believing in me and getting me through the tough times.

I would also like to thank the Superintendent, Principals and Teachers of Parkland School County, and all the parents and children in the research study. Without them, this research would not have been possible.

This work was financially supported by Natural Sciences and Engineering Research Council of Canada, MTI Meta Tech Inc, and Wyeth Nutitionals Incorporated. Vanessa Lien was a recipient of the Natural Science and Engineering Research Council (NSERC) Postgraduate Scholarship, the Alberta Heritage Foundation for Medical Research Health Research Studentship, and the Dietitians of Canada Graduate Memorial Award.

Table of Contents

Chapter 1.
General Introduction..... 1

1.1 Introduction..... 1
1.2 Omega 6 and Omega 3 fatty acids..... 2
1.2.1 Nomenclature..... 2
1.3 Essential Fatty Acids and Metabolic Pathways..... 3
1.4 Dietary Sources..... 7
1.5 Eicosanoids: Fatty Acid Derivatives..... 7
1.6 Excess Essential Fatty Acids..... 8
1.7 Balance Between Omega 6 and Omega 3 Fatty Acids..... 9
1.8 Current Recommendations and Current Nutrient Intakes of Polyunsaturated Fatty Acids..... 12
1.8.1 Nutrient Intakes of LA and LNA..... 12
1.8.2 Nutrient Intakes of AA and DHA..... 13
1.9 Omega 6 and Omega 3 Essentiality..... 16
1.9.1 Importance of AA and DHA: Retinal and Neuro Development..... 16
1.9.2 Synthesis of AA and DHA..... 17
1.9.3 AA and DHA Intakes (mg/kg)..... 18
1.10 Other nutrients of importance: Vitamin E, iron, and Vitamin A..... 19
1.10.1 Vitamin E..... 19
10.1.2 Iron..... 20
10.1.3 Vitamin A..... 22
1.11 Reference List..... 24

Chapter 2.
Research Plan..... 41
2.1 Rationale..... 41
2.2 Research Objectives..... 41
2.3 Hypotheses..... 42
2.4 Chapter Format..... 42

Chapter 3.
Assessment of Docosahexaenoic Acid, Arachidonic Acid, Vitamin A, Vitamin E, and Iron Nutrient Intakes in 4-7 year old Children..... 44
3.1 Introduction..... 44
3.2 Methods..... 46
3.2.1 Subjects..... 46
3.2.2 Dietary Assessment..... 47
3.2.3 Fatty Acid Analysis of Specific Foods..... 47
3.2.4 Statistical Analysis..... 48
3.3 Results..... 48
3.3.1 Subjects..... 48

3.3.2 Dietary Assessment..... 48
Macronutrient, Vitamin and Mineral Intake..... 48
AA and DHA Intake..... 51
Gender and Income Comparison..... 54
3.4 Discussion..... 54
3.4.1 Macronutrient, Vitamin and Mineral Intakes..... 54
3.4.2 Food Assessment Method used to Assess AA and DHA Intakes..... 55
3.4.3 Intakes of AA and DHA..... 56
3.4.4 Inter and Intra Variation in AA and DHA Intakes..... 58
3.4.5 AA and DHA Intakes Compared to Infant Values..... 59
3.4.6 Income Comparison..... 60
3.5 Conclusion..... 60
3.6 Reference List..... 62

Chapter 4.
Consuming a nutritionally complete supplement containing AA and DHA for 7 months improves visual perception in children 4-7 years of age..... 68
4.1 Introduction..... 68
4.2 Methods..... 70
4.2.1 Subjects..... 70
4.2.2 Inclusion and Exclusion Criteria..... 70
4.2.3 Study Design..... 71
4.2.4 Dietary Formula..... 72
4.2.5 Randomization..... 74
4.2.6 Growth..... 74
4.2.7 Diet Assessment..... 75
4.2.8 Visual Function Assessment..... 76
4.2.9 Neurological Assessment..... 77
4.2.10 Health Diary..... 78
4.2.11 Fatty Acid Analysis..... 78
4.2.12 Sample Size..... 79
4.2.13 Statistical Analysis..... 80
4.3 Results..... 81
4.3.1 Subjects..... 82
4.3.2 Formula Intake..... 82
4.3.3 Growth..... 83
4.3.4 Diet Assessment..... 83
Food Records..... 83
DHA Intake Assessed by Food Frequency Questionnaire..... 93
Comparison of DHA Intakes Assessed by Food Frequency Questionnaire and Food Record..... 95
4.3.5 Visual Function Assessments..... 95
4.3.6 Neurological Assessment..... 96
4.3.7 Individual Fatty Acids (AA and DHA in plasma and red blood cell phospholipids)..... 99
4.3.8 Health Diary..... 102

4.4 Discussion..... 102
4.4.1 Diet Assessment..... 102
Macronutrient, Vitamin and Mineral Intakes..... 102
Food Assessment Method used to Assess AA and DHA Intakes..... 104
AA and DHA Intakes..... 105
4.4.2 Visual Function Assessments..... 106
4.4.3 Neurological Assessment..... 107
4.4.4 Individual Fatty Acids (AA and DHA in plasma phospholipids and RBC phospholipids)..... 108
4.5 Conclusion..... 109
4.6 References..... 111

Chapter 5.
Final Summary Discussion and Conclusions..... 119
5.1 General Summary..... 119
5.2 Conclusions..... 120
5.3 Overall Conclusion, Limitations and Future Research..... 121
5.3.1 Study 1..... 121
5.3.2 Study 2..... 122
5.4 References..... 126

Appendix A..... 127
Appendix B..... 128
Appendix C. Food Frequency Questionnaire..... 129
Appendix D..... 131
Appendix E..... 135
References..... 137

List of Tables

Table 1.1 Structure and Name of Fatty Acids..... 4

Table 3.1 Average Daily Nutrient Intake for Children Aged 4-7 Assessed by 3-day Food Records..... 49

Table 3.2 Average Daily Vitamin and Mineral Intake for Children aged 4-7 Assessed by 3-day Food Records..... 50

Table 3.3 Average Daily Fatty Acid Intake for Children aged 4-7 Assessed by 3-day Food Records..... 51

Table 4.1 Weight, Height, and Body Mass Index of Subjects in Control and LCP Groups at 0 and 7 Months..... 83

Table 4.2 Daily Nutrient, Vitamin and Mineral Intake from Food Consumption Assessed using 4-day Food Record at 0 and 7 months of Subjects in Control and LCP Groups..... 86

Table 4.3 Daily Fatty Acid Intake of Food Consumption Assessed Using 4-day Food Record at 0 and 7 Months of Subjects in Control and LCP Groups..... 88

Table 4.4 Comparison of Docosahexaenoic Acid Intake Determined From 4-day Food Record and Food Frequency Questionnaire at 0 and 7 months in Control and LCP Subjects..... 94

Table 4.5 Mean and Standard Deviation of Variables from TVPS-R at 0 and 7 Month Comparisons of Subjects in Control and LCP Groups..... 97

Appendix Tables

Table A.1 Fatty Acid Profile Content of Control and LCP Supplement Per Sachet Serving..... 127
Table B.1 Nutrient Composition of Control and LCP Supplement fed..... 128

List of Figures

Figure 1.2 Essential Fatty Acid Metabolism..... 6

Figure 3.1 Distribution of AA Intake (mg/day) Assessed by 3-day Food Records for Children Aged 4-7..... 52

Figure 3.2 Distribution of DHA Intake (mg/day) Assessed by 3-day Food Records for Children Aged 4-7..... 53

Figure 4.1 Mean Individual Intakes of AA (mg/day) from Food Consumption Assessed by 4-day Food Records at 0 and 7 Months in Control and LCP Subjects..... 91

Figure 4.2 Mean Individual Intakes of DHA (mg/day) from Food Consumption Assessed by 4-day Food Records at 0 and 7 Months in Control and LCP Subjects..... 92

Figure 4.3 DHA Intake (mg/day) plotted versus Median Visual Perceptual age (months) 98

Figure 4.4 AA and DHA level in Plasma Phospholipids in Control and LCP Subjects at 7 Months..... 100

Figure 4.5 AA and DHA level in Phospholipids of Red Blood Cells in Control and LCP Subjects at 7 Months..... 101

Appendix Figures

Figure D.1 Sample HOVT Chart used to Assess Visual Acuity in Children..... 131
Figure D.2 Sample Lang Test used to Assess Stereo Acuity in Children..... 132
Figure D.3 Sample Ishihara Pseudoisochromatic Plate used to Assess Colour Vision in Children 133
Figure D.4 Prism Cover Test used to Assess Strabismus..... 134
Figure E.1 Sample Graphical Representation of Each of the 7 Subsets in the Test of Visual Perceptual Skills (non-motor)- Revised (TVPS-R)..... 135

List of Abbreviations

AA	Arachidonic Acid
AI	Adequate Intakes
AMDR	Acceptable Macronutrient Distribution Ranges
ALA	Linolenic Acid
ANOVA	Analysis of Variance
BMI	Body Mass Index
DHA	Docosahexaenoic Acid
EAR	Estimated Energy Requirements
EFA	Essential Fatty Acids
EPA	Eicosapentaenoic Acid
LA	Linoleic Acid
LBW	Low Birth Weight
LCP	Long Chain Polyunsaturated Fatty Acids
PUFA	Polyunsaturated Fatty Acids
RBC	Red Blood Cell
TVPS-R	Test of Visual Perceptual Skills (non-motor)-Revised

Chapter 1- General Introduction

1.1 Introduction

Long chain polyunsaturated fatty acids (LCP), particularly arachidonic acid (AA) and docosahexaenoic acid (DHA) are important in infant growth and development (Clandinin et al. 1981). These fatty acids are major components of the membranes of the brain and retina (Sastry 1985; Tinoco 1982). Supplementing infant formulas with both AA and DHA or DHA alone, has shown improvement in vision and brain development in both preterm (Birch et al. 1992; Clandinin et al. 2005) and term infants (Agostoni et al. 1995; Birch et al. 2002). Infant formulas in North America now contain AA and DHA (Health Canada 2002; U.S. Food and Drug Administration 2002). Many other countries such as Europe, Middle East, South America, Australia, Japan, Thailand, and some other Asian countries, already have LCP added to many of their preterm and term formulas (Fleith and Clandinin unpublished).

Although the rate of growth slows, the brain and eye continue to develop throughout childhood (Chugani 1998; Dobbing 1972; Oyster 1999). However, there is limited research investigating vision and brain development beyond 2 years of age. This literature review will investigate the role of AA and DHA in retinal and brain development, current dietary intake of these fatty acids in children, and provide an understanding of the importance of LCP in childhood.

1.2 Omega 6 and Omega 3 Fatty Acids

1.2.1 Nomenclature

There are two families of polyunsaturated fatty acids (PUFA): omega 6 and omega 3 fatty acids. Fatty acids are named using four common systems: two abbreviations (n-designation, Δ-designation), systematic name, and trivial name. For the n-designation (also known as the omega (ω) end of the chain), the carbon numbering starts from the methyl end of the fatty acid. This reference system indicates the total number of carbons atoms in the chain, the number of double bonds, and the position or carbon atom number of the first double bond in the chain. For example with 18:2n6 or 18:2ω6, there are 18 total carbon atoms in the chain, there are two double bonds, and the carbon atom number of the first double bond in the chain denoted from the methyl end is 6 (Table 1.1). In the Δ-designation, this system indicates the total number of carbon atoms, the total number of double bonds, and the superscripted numbers after the delta system indicate the position of the carbon atoms numbered from the carboxyl end at which the double bond begins. For example, $18:2\Delta^{9,12}$, denotes that there are 18 carbon atoms in total, there are two double bonds, at the 9 and 12 position, counting from the carboxyl end. Another type of system is the systematic name, which follows the nomenclature from the International Union of Pure and Applied Chemistry (IUPAC-IUB Commission on Biochemical Nomenclature 1974). This system modifies the name of the straight chain hydrocarbon and has the same number of carbon atoms. Using the hydrocarbon name, it removes the final –e, and replaces it with a –oic followed by the word acid. The trivial (common name) is typically derived from the common source of the compound or the source from which it was first isolated and gives no clues to the

structure, and must be memorized if one chooses to use this method of naming a fatty acid (Berdanier 2000).

1.3 Essential Fatty Acids and Metabolic Pathways

PUFA, particularly linoleic acid (18:2n-6, LA) and linolenic acid (18:3n3, ALA), are considered precursors of longer chain omega 6 and omega 3 fatty acids (Connor and Neuringer 1988). The 'parent' PUFA are known as essential fatty acids (EFA) and are necessary in the diet as they cannot be synthesized in humans. Burr and Burr (1929; 1930) were one of the first to describe that fats were essential to the diet through the investigation of a fat-free diet in rats. When fat is excluded in the diet, a deficiency disease develops which in rats results in an early death, unless they are given a curative dose of fat (Burr and Burr 1929; 1930).

Symptoms of EFA deficiency include growth retardation (Caldwell et al. 1972; Evans et al. 1934a, b), impaired reproduction (Evans et al. 1934a, b), kidney damage (Holman 1968), skin (Hansen et al. 1958; T-W-Fiennes et al. 1973) and hair abnormalities (Caldwell et al. 1972; T-W-Fiennes et al. 1973), impaired immune function (Caldwell et al. 1972), premature death (Evans et al. 1934a, b), fatty liver (Holman 1968), degenerative changes in the lung, (Uauy et al. 1989), and increased metabolic rate (Uauy et al. 1989).

Table 1.1 Structure and Name of Fatty Acids

Structure/ Formula	Abbreviation systems		Systematic Name (IUPAC)	Trivial Name
	n-designation (or ω-designation)	Δ-designation		
$CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	18:2n6 (18:2w6)	$18:2\Delta^{9,12}$	9,12-Octadecadienoic	Linoleic
$CH_3CH_2(CH=CHCH_2)_3(CH_2)_7COOH$	18:3n3 (18:3ω3)	$18:3\Delta^{9,12,15}$	9,12,15-Octadecatrienoic	Linolenic
$CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2COOH$	20:4n6 (20:4ω6)	$20:4\Delta^{5,8,11,14}$	5,8,11,14-Eicosatetraenoic	Arachidonic
$CH_3(CH_2CH=CH)_5(CH_2)_3COOH$	20:5n3 (20:5ω3)	$20:5\Delta^{5,8,11,14,17}$	5,8,11,14,17-Eicosapentaenoic	Timnodonic or Eicosapentaenoic
$CH_3(CH_2CH=CH)_6(CH_2)_2COOH$	22:6n3 (22:6ω3)	$22:6\Delta^{4,7,10,13,16,19}$	4,7,10,13,16,19-Docosahexaneoic	Cervonic or Docosahexaneoic

Adapted from King (1996)

More specifically, symptoms of ALA deficiency include impaired visual acuity (Connor et al. 1991; Neuringer et al. 1986; Neuringer et al. 1984), abnormal electroretinogram (Connor et al. 1991; Neuringer et al. 1986), polydispia (Connor et al. 1991; Reisbick et al. 1990), learning impairment (Lamptey and Walker 1976; Yamamto et al. 1988), neurological abnormalities (Holman et al. 1982), growth retardation (Bjerve et al. 1988), and dermatitis (Bjerve et al. 1987a; Bjerve et al. 1989; Bjerve et al. 1987b).

Mammals are unable to make these EFA as they lack the enzymes required to insert double bonds at carbon atoms beyond carbon 9 position (Rivers et al. 1975). These enzymes can be found in plant chloroplasts and are capable of converting non-essential fats, such as oleic acid, into EFA including LA, which is then converted to ALA (Leaf and Weber 1988). Once LA ($18:2\Delta^{9,12}$) and ALA ($18:3\Delta^{9,12,15}$) are ingested in the diet, humans are able to metabolize into longer chain polyunsaturated fatty acids (LCP) using desaturation and elongation reactions. The fatty acid chains are elongated by the enzymatic addition of 2 carbon atoms at the carboxylic end of the chain, and metabolites such as arachidonic acid (AA) from LA, and docosahexaenoic acid (DHA) from ALA is formed (Figure 1.2). For a number of years, it was assumed that the final steps of the synthesis of DHA and docosapentaenoic acid n-6 involved the desaturation at the position 4 by the microsomal acyl-CoA dependent desaturase of 22:5n-3 to 22:6n-3 and 22:4n6 to 22:5n3 (Ferdinandusse et al. 2001; Sprecher et al. 1995). However, several studies have shown that the endoplasmic reticulum does not contain the Δ4 desaturase (Mohammed et al. 1995; Voss et al. 1991; Wang and Anderston 1993) and it is now established that the synthesis of DHA and docosapentaenoic acid n-6 (C22:5n-6) involves partial β-oxidation in the peroxisomes (Sprecher et al. 1995).

When there is an EFA deficiency, eicosatrienoic acid (20:3n9) will be formed from oleic acid (18:1n9) (Mead 1968). Since humans are capable of introducing double bonds in the omega 9 position, omega 9 fatty acids are considered non essential and can be synthesized from other metabolic sources including saturated fats, carbohydrates or ketones (Decsi et al. 1995). This thesis will focus on AA and DHA.

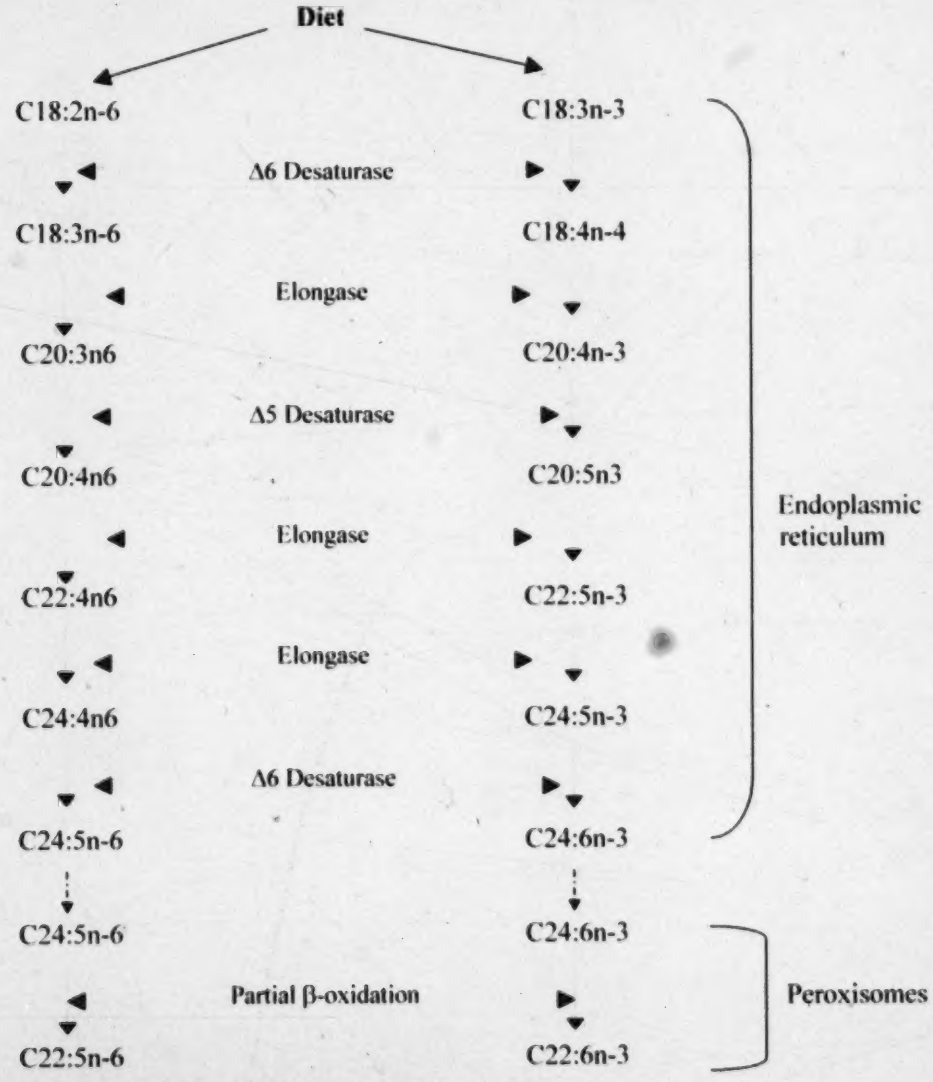


Figure 1.2 Essential Fatty Acid Metabolism
Adapted from Innis (1994)

1.4 Dietary Sources

LA is the most common PUFA in the Western diet and can be found in most seeds and oils of plants such as corn, safflower, soybean, cottonseed, sunflower seed, and peanut oil (U.S. Department of Agriculture 1998). ALA can be found in green leafy vegetables, linseed, soybean, walnuts, flaxeed, canola, and other seed oils (U.S. Department of Agriculture 1998). Dietary sources of AA and DHA include fish, shellfish, liver, poultry, eggs, beef and pork (Exler and Weihrauch 1976, 1977; U.S. Department of Agriculture 1998). Higher levels of DHA are found in fatty fish such as mackerel, salmon, herring. The location and time of year of capture, as well as the way fish is prepared, can all affect the content of n-3 in fish and seafood (Exler and Weihrauch 1976, 1977; Hepburn 1986; Schmidt et al. 2001; Soriguer et al. 1997).

1.5 Eicosanoids: Fatty Acid Derivatives

The LCP derived from the n-6 and n-3 EFA, are precursors of eicosanoids. These are metabolites of 20 carbon fatty acids that consist of prostaglandins, prostacyclins, thromboxanes, and leukotrienes as reviewed by Calder (2001) and Leaf and Weber (1988). When LA is metabolized into eicosatrienoic acid, it serves as a precursor for the prostaglandins 1 series and the leukotrienes 3 series, where as AA is a precursors of series 2 prostanoids (prostaglandin and thromboxane) and 4 series leukotrienes (Calder 2001). When ALA is metabolized into eicosapentaenoic acid (EPA) it forms both the prostaglandin 3 series and leukotriene 5 series (Calder 2001).

Many of the prostaglandins and thromboxanes have antagonistic effects, and different potency levels in the human body. Thromboxanes derived from AA, such as

TXA_2 can affect vasoconstriction/platelet aggregation, whereas TXA_3 derived from EPA performs the same function but at a much lower potency level (Calder 2001). Leukotrienes, specifically LTB_5 (derived from EPA) is 10 fold less potent as a neutrophil chemottractant than LTB_4 (derived from AA), and therefore can be considered less inflammatory (Calder 2001). Eicosanoids derived from AA (2 series) tend be to proinflammatory, proaggregating, vasoconstriction action, and exhibit immunosuppressive properties, whereas the 3 series derived from EPA tend to be antinflammatory, antiaggregating, vasodilatory, antiarythmic action, and immunomodulating properties (Leaf and Weber 1988).

1.6 Excess Essential Fatty Acids

Under oxidative stress, PUFA can be attacked by free radicals and oxidized lipid peroxides which can result in increased oxidative damage and involvement in many human diseases such as atherosclerosis (Halliwell and Chirico 1993). As a result, diets rich in PUFA have increased requirements of vitamin E (Basu and Dickerson 1996). Vitamin E is an antioxidant and helps stabilize free radicals that can be extremely damaging to the body (McDowell 2000; Sokol 1988).

Large intakes of omega 3 may also result in decreased platelet aggregation, increased bleeding time, shift in eicosanoid metabolism (inhibition of AA, replaced by EPA- resulting in the generation of prostacyclins and thromboxanes), immunosuppression (Eritsland 2000; Simopoulos 1991), and changes in myelin configuration and function (Friedman 1980).

1.7 Balance Between Omega 6 and Omega 3 Fatty Acids

The balance of omega 6 and omega 3 fatty acids is dependent on its parent precursors, LA and ALA as these fatty acids compete with oleic acid for binding to the microsomal enzyme system which allows for the further desaturation and chain elongation (Decsi et al. 1995). The competitive inhibition between the two series of fatty acids for the Δ6 and Δ5 is dependent on the absolute amounts of LA and ALA and can be observed in both animal and human studies (Emken et al. 1994; Mohrhauer and Holman 1963; Salem et al. 1999). When rats were fed dietary linolenate at levels equal or exceeding linoleate, Mohrhauer and Holman (1963) found that linolenate competitively inhibited the conversion of linoleate. The number of double bonds in the fatty acid, that is linolenic>linoleic>oleic acid, determines the affinity of the fatty acids to the converting enzyme (Mohrhauer and Holman 1963).

Animal studies and human feeding studies provide evidence that supplementing LA lead to increases in AA in rat plasma membrane phospholipids (Field et al. 1989), whereas supplementing ALA leads to limited or no increase in DHA plasma phospholipids (Clandinin et al. 1997; Layne et al. 1996). Field et al. (1989) supplemented Sprague-Dawley rats with high fat diets (20% wt/wt) for 6 weeks, by increasing the ratio of polyunsaturated/saturated fatty acid ranging from 0.14 to 1.80. This was achieved by increasing LA in the diet (10.7 to 55.9 % wt/wt), while maintaining ALA content to ensure adequate intake of omega 3 fatty acids (Field et al. 1989). Increasing LA content in the diet lead to increase in AA fatty acyl composition of adipocyte plasma membrane phospholipids (%wt/wt) in a dose dependent manner (Field et al. 1989). Supplementation with fish oil (35 mg EPA+DHA/kg body weight/day) in

human adults for 3 months lead to increases in DHA content of total plasma phospholipids compared to when the same subjects were provided with olive oil (35 mg LA/kg body weight/day) or linseed oil (35 mg ALA/kg body weight/day) treatments (Layne et al. 1996). Linseed oil, a source of ALA was not effective in increasing DHA plasma phospholipid levels (Layne et al. 1996). Clandinin et al. (1997) supplemented preterm infants with one of four formulas containing increasing AA and DHA ranging from 0-1.1% (wt/wt of total fatty acids) AA and 0-0.75% (wt/wt of total fatty acids) DHA, while another group was provided with human milk. Providing formula containing increasing levels of AA and DHA resulted in a clear dose response in AA and DHA in total plasma phospholipids (Clandinin et al. 1997). Plasma levels of AA and DHA declined in infants not fed AA and DHA (Clandinin et al. 1997)

Stable isotope studies confirm that providing ALA leads to limited or no increases in DHA plasma phospholipids (Burdge et al. 2002; Hussein et al. 2005). To investigate the conversion of ALA to the n-3 LCP, adult men were administered [U^{13}C] ALA orally with their habitual diet (Burdge et al. 2002). This study showed that the principal products of desaturation of elongation of ALA were EPA and DPA, however there was no evidence of increased ^{13}C enrichment in DHA (Burdge et al. 2002). Similarly, other stable isotope studies, where ALA is labeled in human adults show relatively low conversion of ALA to EPA and DPA, and limited DHA synthesis (Emken et al. 1994; Hussein et al. 2005; Pawlosky et al. 2001; Salem et al. 1999; Vermunt et al. 1999; Vermunt et al. 2000). When subjects were provided with EPA+DHA in the diet, ALA conversion was down-regulated but was not up-regulated by increased ALA consumption (Burdge et al. 2003). Increased ALA intake was associated with increases in EPA and

DPA concentrations but there was no significant effect on DHA concentrations, whereas increased EPA+DHA consumption was associated with lower cumulative concentrations of labeled EPA and DPA, but not DHA (Burdge et al. 2003). This suggests that increased ALA consumption maybe useful means for increasing synthesis of EPA and DPA but not DHA.

The ratio of omega 6 to omega 3 fatty acids are important to consider as competition between these two classes of PUFA can have an affect on the eicosanoids produced as reviewed in Simopoulos (1991). That is, EPA (omega 3 family) compete with AA (omega 6 family) for the production of their respective eicosanoids (Simopoulos 1991). When rats were fed increasing levels of 18:3n3 in the diet, the elongated, desaturated, metabolites of the n-6 series decreased, as the n-3 series increased, as a result of the competitive inhibition of 18:3n3 over 18:2n6 for Δ6 desaturase, leading to a decrease in the prostaglandin 2 series, and decreased levels of AA (Marshall and Johnston 1982). In review articles looking at the ratio of omega 6 to omega 3, Simopoulos (2003; 1991) reports that when humans ingest fish or fish oil, the EPA and DHA from the diet partially replaces n-6 fatty acids such as AA, this increase of omega 3 affects the production of various prostaglandins (decreased PGE_2), thromboxanes (decreased TXA_2, a potent platelet aggregator and vasoconstrictor), and leukotrienes (decreased LTB_4, an inducer of inflammation and powerful inducer of leukocyte chemotaxis and adherence). A diet rich in omega 6 fatty acids shifts the physiological state to one that is prothrombotic and proaggregatory, with increased blood viscosity, vasospasm, and vasoconstriction and decreases in bleeding time (Simopoulos 2003).

Thus, a balance of omega 3 and omega 6 fatty acids are important homeostasis and normal development (Simopoulos 2003).

1.8 Current Recommendations and Nutrient Intakes of Polyunsaturated Fatty Acids

In North America, adequate intakes (AI) of LA and ALA, 10 g/day and 0.9 g/day respectively, have been recommended for children between 4-8 years of age (Institute of Medicine 2002). The acceptable macronutrient distribution ranges (AMDR), for n-6 PUFA are between 5-10% (LA) and n-3 PUFA are 0.6-1.2% (ALA, of which 10% can come from LCP) for children 4-18 years of age (Institute of Medicine 2002). In the current Western diet, the ratio of omega 6:omega 3 have been estimated to be between 10:1 to 20-25:1(Kris-Etherton et al. 2000; Simopoulos 1991).

1.8.1 Nutrient Intakes of LA and ALA

Of the published data available on the intakes of LA and ALA in children, the data is relatively consistent between studies. Jonnalagadda et al. (1995) determined fatty acid intake using data from a 1987-1988 US Nationwide Food Consumption Survey from a three day period (one 24 hour recall, and 2-day food record). The estimated intake of children 6-11 years of age was reported to be 11±0.2 g/ day and 1±0.02 g/day (Jonnalagadda et al. 1995). This was similar to the LA and ALA intakes reported by Allison et al. (1999), who also estimated fat intakes from a national survey (1989-1991 US Continuing Survey of Food Intakes of Individuals) from a three-day period (24 hour recall and 2-day food record), and found that children 3 years and older had intakes of

12.8±0.21g/day and 1.2±0.02g/day respectively. Finnish preschool children 4-7 years of age, had LA and ALA intakes of 9.7±4.1 g/day and 1.3±0.6 g/day respectively, using 3-day food records (Ylonen et al. 1996). Results were similar to that reported by Innis et al. (2004) who found that in Canadian children 3-5 years of age, using a food frequency questionnaire, had intakes of 9.39±0.63 g/day for LA and 1.72±0.17 g/day for ALA. In Australian children, reported intakes of LA and ALA (using a 24-hour recall) were 7.5 g/day and 0.81 g/day for children 4-7 years of age (Meyer et al. 2003).

1.8.2 Nutrient Intakes of AA and DHA

Few studies report AA and DHA intake in either children or adults. Of these studies, the data reported varies. Meyer et al. (2003) reported that AA and DHA intakes in Australian children (aged 4-7, n=799) was 22 mg/day and 47 mg/day using a 24-hour dietary intake record. They also reported that in Australian adults (≥19 years of age, n=10 851), mean and median AA intake was 52 mg/day and 24 mg/day, and for DHA mean and median intake for DHA was 106 mg/day and 15 mg/day. Higher intakes were observed in a study done by Jonnalagadda et al. (1995), where fatty acid intake was determined using data from 1987-1988 US Nationwide Food Consumption Survey from a three day period (one 24 hour recall, and 2-day food record). Intake of children aged 6-11 was 100±0 mg/day for 18:4+AA and 100±0 mg/day for 20:5+DHA. Similar results were found by Allison et al. (1999), who estimated fatty acid intake from the 1989-1991 US Continuing Survey of Food Intakes of Individuals from a three-day period (24 hour recall and 2-day food record). Allison et al. (1999) reported that fatty acid intake for those 3 years and older was 100±10 mg/day for 18:4+AA and 100±10 g/day for

20:5+DHA. Innis et al.(2004) reported AA (226±17 mg/day) and DHA (96±14 mg/day) intakes in 3-5 year old Canadian children using a food frequency questionnaire.

Raper et al. (1992) investigated annual per capita food use data in the US food supply and found that the DHA levels have increased from 69 to 78 mg/capita/day between 1935-1939 and 1985 and was the result of increased use of canned tuna, gamefish, and poultry. Kris-Etherton (2000) estimated that DHA per capita disappearance from fish in the United States was 0.25 g/day and worldwide the average was 0.23 g/day.

Geographic location where nutrient assessments were completed is likely one of the reasons why wide variability of AA and DHA intake are reported. Many of the nutrient assessments were national surveys (Allison et al. 1999; Jonnalagadda et al. 1995; Meyer et al. 2003) which provide the mean intakes of AA and DHA for all subjects in the population. Although it is important to have an idea of the intakes of AA and DHA consumed by a population, it does not identify groups in the population that have lower intakes of these fatty acids. In the study by Innis et al.(2004), DHA intake was expected be to higher than the present study, as the preschool children studied were from the Vancouver Costal Health Authority Region, British Columbia, Canada, an area where seafood is readily available.

The food assessment method used to assess AA and DHA intake may be another reason why wide variability of AA and DHA intake are reported. Allison et al. (1999) and Jonnalagadda et al. (1995) collected food intake over a three day period (one 24 hour recall, and 2-day food record). Using a 7-day weighted food record maybe considered a more precise method available for estimating usual food and/or nutrient intakes of

individuals, assuming respondents do not change their usual eating pattern (Gibson 1990). However this method would not have been feasible due to the large number of participants in the studies conducted by Allison et al. (1999) and Jonnalagadda et al. (1995). A 24-hour recall method was used by Meyer et al. (2003) however a single day is not representative of the usual fat consumption of an individual (Block 1982; Pekkarinen 1970). Food frequency questionnaires provide an inferior quantitative estimate of intake as this method does not usually define information on a specific food items consumed such as exact portion sizes, food preparation methods, brand name, and packaging information (Allison et al. 1999; Briefel et al. 1992; Sempos 1992). Innis et al. (2004) used a food frequency questionnaire, and considered this limitation and collected data on specific foods (e.g. frequency food was eaten, portion sizes, brand name or place of purchase, method of preparation, use of fat-reduced foods, types of margarines, shortenings, and other fats and oils), therefore, AA and DHA nutrient estimates are likely to be representative of the preschool children studied. Raper et al.(1992) and Kris-Etherton (2000), used per capita food use data or per capita disappearance data to estimate DHA intakes, however, this method overstates actual consumption as it does not account for spoilage and waste, and is not a direct measure of the quantity of food actually consumed (Economic Research Service United States Department of Agriculture 2004).

1.9 Omega 6 and 3 Essentiality

1.9.1 Importance of AA and DHA: Retinal and Neuro Development

Long chain polyunsaturated fatty acids (LCP), specifically arachidonic acid (20:4n-6, AA) and docosahexaneoic acid (22:6n-3, DHA) play an important role in infant growth and development (Clandinin et al. 1981). These fatty acids are major components of the membranes of the brain and retina (Sastry 1985; Tinoco 1982). During infant development, there is rapid accretion of AA and DHA during the last trimester of gestation (Clandinin et al. 1980). Infants born prematurely are deprived of some of this *in utero* accretion of AA and DHA unless they are fed AA and DHA (Clandinin et al. 1982). When preterm infants were supplemented with DHA, there was improvement in visual acuity (Birch et al. 1992; Carlson et al. 1993) and speed of information processing (Carlson and Werkman 1996; Werkman and Carlson 1996) was observed. In a recent double-blind multi-centre study, feeding preterm infants (n=361) formula containing AA and DHA for 118 weeks resulted in enhanced growth and higher Bayley mental and psychomotor development scores than control infants that were provided with an unsupplemented formulas (Clandinin et al. 2005). In term infants, AA and DHA have also been shown to improve vision (Birch et al. 2002; Birch et al. 1998; Hoffman et al. 2003), and brain development (Agostoni et al. 1995; Birch et al. 2000; Birch et al. 2002).

Evidence from animal studies support that DHA is essential: when developing monkeys were fed diets deficient in α-linolenic acid (18:3 n-3, ALA), an essential fatty acid and precursor of DHA, this resulted in decreased DHA levels in the retina and cerebral cortex and impaired visual acuity and neuronal development (Neuringer et al. 1986; Neuringer et al. 1984). There is limited research investigating vision and brain

development beyond 2 years of age, even though neurological growth continues into childhood.

1.9.2 Synthesis of AA and DHA

There is unequivocal evidence that support LA and ALA are essential in the diet of mammals, including humans who lack the ability to insert double bonds at the n-3 and n-6 position of a fatty acid. These EFA, (LA and ALA) are necessary for the desaturation and elongation of these fatty acids to their metabolites, AA and DHA.

Using radiochemical assay, Poisson et al. (1993) confirmed that Δ6 and Δ5 desaturase activities was present in human liver of neonates. Similarly De Gomez Dumm and Brenner (1975) confirmed that desaturase activities were present in human adult liver microsomes. Using fetal pigs (a model analogous to the human for accretion of lipid in the brain (Purvis et al. 1982), Purvis et al. (1983) found that the fetal liver and brain have the ability to elongate and desaturate EFA to their long chain homologues. Synthesis rates of chain elongation-desaturation products in the brain was the greatest during the newborn period, whereas the metabolic function in the liver was greatest in the postnatal stages of life (Purvis et al. 1983). Furthermore, a significant increase in the Δ5 desaturase activity resulting in increases in synthesis of C20 and C22 homologues of EFA was observed during the last half of gestation (Clandinin et al. 1985). In an *in vitro* study, Moore et al. (1991) found that astrocytes (not neurons) were capable of producing AA and DHA from its precursor essential fatty acids, these LCP are then released within the brain for uptake and sequestration by neurons.

Recent studies using stable isotope have suggested that preterm and term infants have the ability to convert LA to AA, and ALA to DHA (Carnielli et al. 1996; Salem et al. 1996; Sauerwald et al. 1996; Szitanyi et al. 1999). However, controversy still exists to whether the contribution of de novo synthesis of these LCP from their dietary precursors is sufficient to support the levels necessary for whole body utilization in a growing infant.

1.9.3 AA and DHA Intakes (mg/kg)

The intakes of AA and DHA can be compared to the intakes by infants consuming mothers milk to infants consuming AA and DHA containing formulas. To estimate AA and DHA (mg/kg) intake in infants, the following assumptions can be made: energy requirement of the infant is 100-120 kcal/kg and human milk provides ~55% of calories from fat. Using the AA and DHA levels in human milk reported earlier (Clandinin et al. 1997), infant intake level of AA range from 33-40 mg/kg and DHA intake range from 18.3-22 mg/kg. Using the AA and DHA levels provided in infant formula (for example: Enfamil A+ (Canadian brand) or Enfamil ® Lipil ® with Iron (US brand) (Mead-Johnson Nutritionals, Evansville, IN)), with assumed energy requirements of an infant is 100-120 kcal/kg, infant intake level of AA ranged from 34-41.2 mg/kg and DHA level ranged from 17.1-20.6 mg/kg. Innis et al. (2004), found that in children 3-5 years of age (assumed weight of a child is 16 kg) AA and DHA intake is 14 mg/day and 6.0 mg/day respectively.

The grey matter of the brain, where DHA accumulates in large amounts during growth and development (Sastry 1985; Sinclair and Crawford 1972), continuously increases from birth through 5 years of age (Levitt 2003). A child's brain weighs 90% of

the adult value by five years of age, and reaches the full weight of an adult brain at 10-12 years of age (Paus et al. 2001). Synaptogenesis, a period of growth spurts in the brain, peaks at eight months and reaches adult values by 11 years (Garey 1984; Garey 1983; Huttenlocher et al. 1982).

Although the rate of growth slows, the brain and eye continue to develop throughout childhood (Chugani 1998; Dobbing 1972; Oyster 1999), thus insufficient supply of AA and DHA may have a negative impact on the retinal and neuronal development in children during the early years of life. Omega-3 and omega-6 fatty acids are necessary for development in both preterm and term infants and the nutritional importance of these fatty acids may extend to childhood. However, no studies have investigated AA and DHA supplementation in children beyond 2 years of age.

This thesis will determine the dietary intakes of AA and DHA by children, then supplement those children (who consume low intakes of DHA) with AA and DHA to determine if the supplementation will increase blood lipid levels and improve visual perception in children over a period of 7 months.

1.10 Other Nutrients of Importance: Vitamin E, Iron, and Vitamin A

1.10.1 Vitamin E

Alpha-tocopherol, the active form of vitamin E, is an important antioxidant that prevents peroxidation of polyunsaturated fatty acids (Dreyfus and Geel 1981). Under oxidative stress, PUFA can be attacked by free radicals and oxidized lipid peroxides which can result in increased oxidative damage and may be involved in many human diseases including atherosclerosis (Halliwell and Chirico 1993). As a result, those

consuming diets rich in PUFA have increased requirements of vitamin E intake (Basu and Dickerson 1996). Vitamin E is an antioxidant and helps stabilize free radicals are potentially damaging to the body (McDowell 2000; Sokol 1988). In humans, prolonged deficiency in vitamin E can result in neurological dysfunction, thus confirming that this vitamin necessary for maintenance and integrity of the human nervous system and skeletal muscle (Sokol 1988). In animals, vitamin E deficiency also leads to the degeneration of the nervous system and skeletal muscle and has been shown to result in poor learning ability and poor memory retention after oxidative stress in young rats (Fukui et al. 2001; Sokol 1988). Both young animals (Bourre et al. 1988), and newborn infants, (Friedman 1980) are sensitive to vitamin E deficiency. In infants, especially low birth weight infants (LBW) are born with insufficient stores of vitamin E, contributed to by low amount of fat deposits in which the vitamin is stored (Friedman 1980). The plasma vitamin E concentration of a newborn infant is one third that of adults, and even lower in the LBW infant (Friedman 1980). Since the LBW infant has limited capacity to absorb fat and fat soluble vitamins, compared to a full term infant, when the infant is provided with a formula containing PUFA, the dietary requirement for vitamin E increases with the increasing amount of dietary PUFA supplied (Friedman 1980).

10.1.2 Iron

Iron is important micronutrient in children, as iron deficiency during key developmental stages has a negative impact on long-term development and behavior as reviewed in Grantham-McGregor and Ani (1999) and Beard and Connor (2003).

Iron deficiency also influences fatty acid metabolism. Stearoyl Coenzyme A (CoA) desaturase is an enzyme that converts palmitic acid (16:0) to palmitoleic acid (16:1n-7) and stearic acid (18:0) to oleic acid (18:1n-9) (Strittmatter et al. 1974). The Stearoyl CoA desaturase enzyme complex consists of NADH cytochrome B5 reductase, cytochrome B5, lipids and the terminal desaturase enzyme is a non-heme iron protein (Strittmatter et al. 1974). As in the stearoyl CoA desaturase, one atom of iron per molecule of enzyme is present in each terminal protein of the Δ6 desaturase complex, (Okayasu et al. 1981), which catalyzes the initial step in the metabolism of the CoA derivatives of LA and ALA to LCP (Cunnane and McAdoo 1987). Presence of iron deficiency affects the synthesis of activity of Δ6 desaturase since it is present in every end protein of the Δ6 desaturase enzyme complex (Okayasu et al. 1981).

Animal and human studies support that iron depletion influences activity or synthesis of the Δ6 desaturase enzyme thus altering EFA metabolism and tissue composition. When young male rats were fed a fat-free low iron diet, decrease in the relative levels of 16:1 to 16:0 and 18:1 to 18:0 in the lipids of tissues were related to decreases in stearoyl CoA desaturase activity (Rao et al. 1980). Similarly, rats fed a moderately low intake of iron resulted in impaired EFA metabolism and altered EFA composition of plasma, erythrocytes, and liver lipids (Cunnane and McAdoo 1987). Oloyede et al. (1992) showed that low iron status aggravated signs of EFA deficiency resulting in decreases in growth, brain weight, and altered brain and lipid fatty acid composition in rats. Similarly, protein-energy malnourished children who were severely anemic were also EFA deficient (Chen and Dickerman 1985). In children 6-11 years of age, iron intervention of 15 weeks lead to an increase in the percentage of total n-3 fatty

acids in the erythrocyte membranes (phosphatidylcholine and phosphatidylethanolamine) in the iron deficient group comparable to the levels in the control group (Smuts et al. 1995).

10.1.3 Vitamin A

Vitamin A is important in the visual process of the retina, and is necessary for night vision and colour perception as reviewed in Basu and Dickerson (1996). Deficiencies in vitamin A may be related with abnormalities in iron metabolism (Mejia et al. 1979), and an association of low hemoglobin and low plasma iron with low plasma retinol has been reported in children (Mejia et al. 1977). Short term supplementation of vitamin A for a period of 2 weeks in children with symptoms of conjunctival xerosis (vitamin A deficiency), lead to an increase in retinol, retinol-binding protein, hemoglobin, hematocrit, serum iron, and saturation of transferrin compared to the unsupplemented group (Bloem et al. 1990). Similarly, xerophthalmic children were supplemented with vitamin A, which led to increases in plasma ferritin, moderate increase in plasma transthyretin, and an increase in hemoglobin in children with low baseline hemoglobin (Semba et al. 1992). These studies suggest that vitamin A deficiency may impair utilization of iron, therefore, supplementing vitamin A in those deficient in vitamin A, may improve the hematological indicators of iron status (Bloem et al. 1990; Semba et al. 1992). When anemic children where supplemented with both vitamin A and iron, there was an improvement in hematological and iron nutrition indicators (including total iron binding capacity and serum ferritin) compared to children who were only supplemented with either vitamin A or iron (Mejia and Chew 1988).

This thesis will determine the dietary intakes of AA and DHA in children. Since vitamin E, iron, and vitamin A are important in normal development and growth, these nutrients will also be assessed in the children to ensure that these nutrients meet the recommended estimated average requirements (EAR suggested by the Institute of Medicine (2000; 2001).

This chapter has focused on the role of AA and DHA in retinal and brain development, current dietary intake of these fatty acids in children, and provided an understanding of the importance of LCP in childhood.

1.11 References

Agostoni C, Trojan S, Bellu R, Riva E, Giovannini M (1995) Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: the role of long-chain polyunsaturated fatty acids. Pediatric Research 38:262-266

Allison DB, Egan K, Barraj LM, Caughman C, Infante M, Heimbach JT (1999) Estimated intakes of trans fatty and other fatty acids in the US population. Journal of the American Dietetic Association 99:166-174

Basu T, Dickerson J (1996) Vitamins in human health and disease. Cab International, Wallingford, Oxon

Beard JL, Connor JR (2003) Iron status and neural functioning. Annual Review of Nutrition 23:41-58

Berdanier CD (2000) Advanced Nutrition: Macronutrients, 2nd edn. CRC Press, Boca Raton

Birch EE, Birch DG, Hoffman DR, Uauy R (1992) Dietary essential fatty acid supply and visual acuity development. Investigative Ophthalmology and Visual Science 33:3242-3253

Birch EE, Garfield S, Hoffman DR, Uauy R, Birch DG (2000) A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Developmental Medicine and Child Neurology 42

Birch EE, Hoffman DR, Castaneda YS, Fawcett SL, Birch DG, Uauy RD (2002) A randomized controlled trial of long-chain polyunsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age. American Journal of Clinical Nutrition 75:570-80

Birch EE, Hoffman DR, Uauy R, Birch DG, Prestidge C (1998) Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. Pediatric Research 44:201-209

Bjerve K, Thoresen L, Borsting S (1988) Linseed and cod liver oil induce rapid growth in a 7-year old girl with n-3 fatty acid deficiency. Journal of Parenteral and Enteral Nutrition 12:512-525

Bjerve KS, Fischer S, Alme K (1987a) Alpha-linolenic acid deficiency in man. Effect of ethyl linolenate on fatty acid composition and biosynthesis of eicosanoids. American Journal of Clinical Nutrition 46:570-576

Bjerve KS, Fischer S, Wammer F, Egeland T (1989) Alpha-Linolenic acid and long-chain omega-3 fatty acid supplementation in three patients with omega-3 fatty acid deficiency: effect on lymphocyte function, plasma and red cell lipids, and prostanoid formation. American Journal of Clinical Nutrition 49:290-300

Bjerve KS, Lovold MI, Thoresen L (1987b) Alpha-linolenic acid and long chain unsaturated n-3 fatty acid requirement in man. American Journal of Clinical Nutrition 45:66-77

Block G (1982) A review of validations of dietary assessment methods. American Journal of Epidemiology 115:492-505

Bloem MW, Wedel M, van Agtmaal EJ, Speek AJ, Saowakontha S, Schreurs WH (1990) Vitamin A intervention: short-term effects of a single, oral, massive oral on iron metabolism. American Journal of Clinical Nutrition 51:76-9

Bourre J-M, Bonneil M, Dumont O, Piciotti M, Nalbone G, Lafont H (1988) High dietary fish oil alters the brain polyunsaturated fatty acid composition. Biochimica et Biophysica Acta 960:458-461

Briefel R, Flegal K, Winn D, Loria C, Johnson C, Sempos CT (1992) Assessing the nation's diet: limitations of the food frequency questionnaire. Journal of the American Dietetic Association 92:959-962

Burdge G, Finnegan YE, Minihane AM, Williams CM, Wootton SA (2003) Effect of altered n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [13C]alpha-linolenic acid to longer-chain fatty acids and partitioning towards b-oxidation in older men. British Journal of Nutrition 90:311-321

Burdge G, Jones AE, Wootton SA (2002) Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. British Journal of Nutrition 88:355-363

Burr GO, Burr MM (1929) A new deficiency disease produced by the rigid exclusion of fat from the diet. The Journal of Biological Chemistry 82:345-367

Burr GO, Burr MM (1930) On the nature and role of the fatty acids essential in nutrition. The Journal of Biological Chemistry 86:587-621

Calder PC (2001) Polyunsaturated fatty acids, inflammation, and immunity. Lipids 36:1007-1024

Caldwell M, Jonsson H, Othersen H (1972) Essential fatty acid deficiency in an infant receiving prolonged parenteral alimentation. Journal of Pediatrics 81:894-898

Carlson SE, Werkman SH (1996) A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until two months. Lipids 31:85-90

Carlson SE, Werkman SH, Rhodes P, Tolley E (1993) Visual acuity development in healthy preterm infants: effect of marine-oil supplementation. American Journal of Clinical Nutrition 58:35-42

Carnielli VP, Wattimena DJ, Luijendijk IH, Boerlage A, Degenhart HJ, Sauer PJ (1996) The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and linolenic acids. Pediatric Research 40:169-174

Chen S, Dickerman S (1985) Iron, thyroid hormone and essential fatty acid status of Honduran preschoolers. Nutrition Research 5:21-30

Chugani HT (1998) A critical period of brain development: studies of cerebral glucose utilization with PET. Preventive Medicine 27:184-188

Clandinin M, Chappell J, Heim T (1982) Do low weight infants require nutrition with chain elongation-desaturation products of essential fatty acids? Progress in Lipid Research 20:901-904

Clandinin M, Chappell J, Heim T, Swyer P, Chance G (1981) Fatty acid utililzation in perinatal de novo synthesis. Early Human Development 5:355-366

Clandinin M, Chappell J, Leong S, Heim T, Swyer P, Chance G (1980) Intraunterine fatty acid accretion rates in human brain: implications for fatty acid requirements. Early Human Development 4:121-129

Clandinin M, Van Aerde J, Parrott A, Field C, Euler A, Lien E (1997) Assessment of the efficacious dose of arachidonic and docosahexaenoic acids in preterm infant formulas: fatty acid composition of erythrocyte membrane lipids. Pediatric Research 42:819-825

Clandinin MT, Van Aerde JE, Merkel KL, Harris CL, Springer MA, Hansen JW, Diersen-Schade D (2005) Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. The Journal of Pediatrics

Clandinin MT, Wong K, Hacker RR (1985) Synthesis of chain elongation-desaturation products of linoleic acid by liver and brain microsomes during development of the pig. Biochemical Journal 226:305-309

Connor W, Neuringer M, Reisbick S (1991) Essentiality of w3 fatty acids: evidence from the primate model and implications for human nutrition. In: Simopoulos A, Kifer R, Martin R, Barlow S (eds) Health Effects of w3 polyunsaturated fatty acids in seafoods. World Review of Nutrition and Dietietics, vol 66. Karger, Basel, pp 118-132

Connor WE, Neuringer M (1988) Importance of dietary omega-3 fatty acids in retinal function and brain chemistry. In: Morley JE, Sterman MB, Walsh JH (eds) Nutritional modulation of neural function. Academic Press Inc., San Diego, California

Cunnane S, McAdoo (1987) Iron intake influences essential fatty acid and lipid composition of rat plasma and erythrocytes. Journal of Nutrition 117:1514-1519

De Gomez Dumm I, Brenner R (1975) Oxidative desaturation of alpha-linolenic, linoleic, and stearic acids by human liver microsomes. Lipids 10:315-317

Decsi T, Thiel I, Koletzko B (1995) Essential fatty acids in full term infants fed breast milk or formula. Archives of Disease in Childhood 72:F23-F28

Dobbing J (1972) Vulnerable periods in of brain development. In: K E, Knight J (eds) Lipids, Malnutrition and the developing brain. Elsevier, Amsterdam, pp 9-20

Dreyfus PM, Geel SM (1981) Chapter 33: Vitamin and Nutritional Deficiencies. In: Siegel GJ, Albers RW, Agranoff BW, Katzman R (eds) Basic Neurochemistry. Little, Brown and Company, Boston

Economic Research Service United States Department of Agriculture (2004) Food consumption: food supply and use. http://www.ers.usda.gov/Data/FoodConsumption/, vol 2004

Emken E, Adlof R, Gulley RM (1994) Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochimica et Biophysica Acta 1213:277-288

Eritsland J (2000) Safety considerations of polyunsaturated fatty acids. American Journal of Clinical Nutrition 71:197-201

Evans H, Lepkovsky S, Murphy E (1934a) Vital need of the body for certain unsaturated fatty acids. V. Reproduction and lactation upon diets containing saturated fatty acids as their sole sources of energy. The Journal of Biological Chemistry 106:441-444

Evans H, Lepkovsky S, Murphy E (1934b) Vital need of the body for certain unsaturated fatty acids. IV. Reproduction and lacation upon fat-free diets. The Journal of Biological Chemistry 106:431-440

Exler J, Weihrauch JL (1976) Comprehensive evaluation of fatty acids in foods. VIII. Finfish. Journal of the American Dietetic Association 69:243-248

Exler J, Weihrauch JL (1977) Comprehensive evaluation of fatty acids in foods. Journal of the American Dietetic Association 71:518-521

Ferdinandusse S, Denis S, Mooijer PA, Zhang Z, Reddy JK, Spector AA, Wanders RJ (2001) Identification of the peroxisomal b-oxidation enzymes involved in the biosynthesis of docosahexaenoic acid. Journal of Lipid Research 42:1987-1995

Field CJ, Toyomizu M, Clandinin MT (1989) Relationship between dietary fat, adiopcyte membrane composition and insulin binding in the rat. Journal of Nutrition 119:1483-1489

Fleith M, Clandinin MT (unpublished) Dietary PUFA for preterm and term infants: review of clinical studies.

Friedman Z (1980) Essential fatty acids revisited. American Journal of Diseases of Children 134:397-408

Fukui K, Onodera K, Shinkai T, Suzuki S, Urano S (2001) Impairment of learning and memory in rats caused by oxidative stress and aging, and changes in antioxidative defense systems. Annals of the New York Academy of Sciences 928:168-175

Garey L (1984) Structural development of the visual system of man. Human Neurobiology 3:75-80

Garey LJ (1983) Development of visual system- comparison of monkey and man. Acta Morphologica Academiae Scientiarum Hungaricae 31:27-38

Gibson R (1990) Principles of Nutritional Assessment. Oxford University Press, New York

Grantham-McGregor S, Ani C (1999) The role of micronutrients in psychomotor and cognitive development. British Medical Bulletin 55:511-527

Halliwell B, Chirico S (1993) Lipid peroxidation: its mechanism, measurement, and significance. American Journal of Clinical Nutrition 57:715S-725S

Hansen A, Haggard M, Boelsche A, Adam D, Wiese H (1958) Essential fatty acids in infant nutrition. III. Clinical Manifestations of linoleic acid deficiency. Journal of Nutrition 66:565- 576

Health Canada FP (2002) Dhasco® and Arasco® as sources of docosahexaenoic acid and arachidonic acid in infant formulas. http://www.hc-sc.gc.ca/food-aliment/mh-dm/otb-bba/nfi-ani/e_dhasco.html, vol 2005

Hepburn FN (1986) Provisional tables on the content of omega-3 fatty acids and other fat components of selected foods. Journal of the American Dietetic Association 86:788-793

Hoffman DR, Birch EE, Castaneda YS, Fawcett SL, Wheaton DH, Birch DG, Uauy R (2003) Visual function in breast-fed term infants weaned to formula with or without long-chain polyunsaturates at 4 to 6 months: a randomized clinical trial. Journal of Pediatrics 142:669-677

Holman R, Johnson S, Hatch T (1982) A case of human linolenic acid deficiency involving neurological abnormalities. American Journal of Clinical Nutrition 35:617-623

Holman RT (1968) Essential fatty acid deficiency. Progress in the Chemistry of Fats and other Lipids 9:275-348

Hussein N, Ahmad A, Wilkinson P, Leach C, Griffin BA, Millward DJ (2005) Long-chain conversion of [13C]linoleic acid and alpha-linolenic acid in response to

marked changes in their dietary intake in men. Journal of Lipid Research 46:269-280

Huttenlocher PR, de Courten C, Garey LJ, Van Der Loos H (1982) Synaptogenesis in human visual cortex- evidence for synapse elimination during normal development. Neuroscience Letters 33:247-252

Innis SM (1994) The 1993 Borden Award Lecture. Fatty acid requirements of the newborn. Canadian Journal Physiology Pharmacology 72:1483-1492

Innis SM, Vaghri Z, King DJ (2004) n-6 Docosapentaenoic acid is not a predictor of low docosahexaenoic acid status in Canadian preschool children. American Journal of Clinical Nutrition 80:768-73

Institute of Medicine (2000) Dietary Reference Intakes for vitamin C, vitamin E, selenium and carotenoids. National Academy Press, Washington, DC

Institute of Medicine (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press, Washington, DC

Institute of Medicine (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). National Academies Press, Washington, DC

IUPAC-IUB Commission on Biochemical Nomenclature (1974) The nomenclature of lipids, Appendix 1. In: Burton R, Guena F (eds) Fundamentals of Lipid Chemistry. BI-Science Publications Division, Webster Groves, MO

Jonnalagadda SS, Egan KS, Heimbach JT, Harris SS, PM K-E (1995) Fatty acid consumption pattern of Americans: 1987-1988 USDA Nationwide Food consumption survey. Nutrition Research 15:1767-1781

King IB (1996) Lipids in foods: chemistry and nomenclature. In: Spiller GA (ed) Handbook of Lipids in Human Nutrition. CRC Press, Boca Raton, pp 3-11

Kris-Etherton P, Taylor DS, Yu-Poth S, Huth P, Moriarty K, Fishell V, Hargrove RL, Zhao G, Etherton TD (2000) Polyunsaturated fatty acids in the food chain in the United States. American Journal of Clinical Nutrition 71:179-188

Lamptey M, Walker B (1976) A possible essential role of dietary linolenic acid in the development of the young rat. Journal of Nutrition 106:86-93

Layne KS, Goh YK, Jumpsen JA, Ryan EA, Chow P, Clandinin MT (1996) Normal subjects consuming physiological levels of 18:3(n-3) and 20:5(n-3) from flaxseed or fish oils have characteristic differences in plasma lipid and lipoprotein fatty acid levels. Journal of Nutrition 126:2130-2140

Leaf A, Weber PC (1988) Cardiovascular effects of n-3 fatty acids. New England Journal of Medicine 318:549-557

Levitt P (2003) Structural and functional maturation of the developing primate brain. Journal of Pediatrics 143:S35-45

Marshall LA, Johnston PV (1982) Modulation of tissue prostaglandin synthesizing capacity by increased ratios of dietary alpha-linolenic acid to linoleic acid. Lipids 17:905-913

McDowell LR (2000) Vitamins in animal and human nutrition, 2nd edn. Iowa State University Press, Ames, Iowa

Mead J (1968) The metabolism of the polyunsaturated fatty acids. Progress in the Chemistry of Fats and other Lipids. 9:161- 192

Mejia L, Hodges R, Arroyave G, Viteri F, Torun B (1977) Vitamin A deficiency and anemia in Central American children. American Journal of Clinical Nutrition 30:1175-1184

Mejia LA, Chew F (1988) Hematological effect of supplementing anemic children with vitamin A alone and in combination with iron. American Journal of Clinical Nutrition 48:595-600

Mejia LA, Hodges R, Rucker R (1979) Clinical signs of anemia in vitamin A-deficient rats. American Journal of Clinical Nutrition 32:1439-1444

Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair A, Howe PR (2003) Dietary intakes and food sources of omega 6 and omega 3 polyunsaturated fatty acids. Lipids 38:391-398

Mohammed B, Sankarappa S, Geiger M, Sprecher H (1995) Reevaluation of the pathway for the metabolism of 7, 10, 13, 16- docosatetraenoic acid to 4, 7, 10, 13, 16- docoxapentaenoic acid in rat liver. Archives of Biochemistry and Biophysics 317:179-184

Mohrhauer H, Holman RT (1963) Alteration of the fatty acid composition of brain lipids by varying levels of dietary essential fatty acids. Journal of Neurochemistry 10:523-530

Moore SA, Yoder E, Murphy S, Dutton GR, Spector AA (1991) Astrocytes, not neurons, produce docosahexaenoic acid (22:6w-3) and arachidonic acid (20:4w-6). Journal of Neurochemistry 56:518-524

Neuringer M, Connor WE, Lin DS, Barstad L, Luck S (1986) Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina and brain in rhesus monkeys. Proceedings of the National Academy of Sciences of the United States of America 83:4021-5

Neuringer M, Connor WE, Petten CV, Barstad L (1984) Dietary omega-3 fatty acid deficiency and visual loss in infant rhesus monkeys. The Journal of Clinical Investigation 73:272-276

Okayasu T, Nagao M, Ishibashi T, Imai Y (1981) Purification and partial characterization of linoleoyl-CoA Desaturase from rat liver microsomes. Archives of Biochemistry and Biophysics 206:21-28

Oloyede O, Folayan A, Odutuga A (1992) Effects of low-iron status and deficiency of essential fatty acids on some biochemical constituents of rat brain. Biochemistry International 27:913-922

Oyster CW (1999) The Human Eye- Structure and Function. Sinauer Associates Inc, Sunderland, Massachusetts

Paus T, Collins D, Evans A, Leonard G, Pike B, Zijdenbos A (2001) Maturation of white matter in human brain: a review of magnetic resonance studies. Brain Research Bulletin 54:255-266

Pawlosky RJ, Hibbeln JR, Novotny JA, Salem NJ (2001) Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. Journal of Lipid Research 42:1257-1265

Pekkarinen M (1970) Methodolgy in the collection of food consumption data. World Review of Nutrition and Dietetics 12:145-171

Poisson J-P, Dupuy R-P, Sarda P, Descomps B, Narce M, Rieu D, Crastes de Paulet A (1993) Evidence that liver microsomes of human neonates desaturate essential fatty acids. Biochimica et Biophysica Acta 1167:109-113

Purvis JM, Clandinin MT, Hacker RR (1982) Fatty acid accretion during perinatal brain growth in the pig. A model for fatty acid accretion in human brain. Comparative Biochemistry and Physiology 72B:195-199

Purvis JM, Clandinin MT, Hacker RR (1983) Chain elongation-desaturation of linoleic acid during the development of the pig implications for the supply of polyenoic fatty acids to the developing brain. Comparative Biochemistry and Physiology 75B:199-204

Rao GA, Manix M, Larkin EC (1980) Reduction of essential fatty acid deficiency in rats fed a low iron fat free diet. Lipids 15:55-60

Raper NR, Cronin FJ, Exler J (1992) Omega-3 fatty acid content of the US food supply. Journal of the American College of Nutrition 11:304-308

Reisbick S, Neuringer M, Hasnain R, Connor W (1990) Polydipsia in rhesus monkeys deficient in omega 3 fatty acids. Physiology and Behavior 47:315-323

Rivers J, Sinclair A, Crawford M (1975) Inability of the cat to desaturate essential fatty acids. Nature 258:171-173

Salem NJ, Pawlosky RJ, Wegher B, Hibbeln JR (1999) In vivo conversion of linoleic acid to arachidonic acid in human adults. Prostaglandins, Leukotrienes and Essential Fatty Acids 60:407-410

Salem NJ, Wegher B, Mena P, Uauy R (1996) Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. Proceedings of the National Academy of Sciences of the United States of America 93:49-54

Sastry P (1985) Lipids of nervous tissue: composition and metabolism. Progress in Lipid Research 24:66-176

Sauerwald T, Hachey D, Jensen C, Chen H, Anderson RE, Heird W (1996) Effect of dietary alpha-linolenic acid intake on incorporation of docosahexaenoic and arachidonic acids into plasma phospholipids of term infants. Lipids 31:S131-S135

Schmidt EB, Christensen JH, Aardestrup I, Madsen T, Riahi S, Hansen VE, Skou HA (2001) Marine n-3 fatty acids: basic features and background. Lipids 36:S65-S68

Semba R, Muhilal M, West KP, Winget M, Natadisastra G, Scott A, Sommer A (1992) Impact of vitamin A supplementation on hematological indicators of iron metabolism and protein status in children. Nutrition Research 12:469-478

Sempos CT (1992) Invited commentary: some limitations of semiquantitative food frequency questionnaires. American Journal of Epidemiology 135:1127-1132

Simopoulos A (2003) Importance of the ratio of omega-6/omega-3 essential fatty acids: evoluntionary aspects. In: Simopoulos A, Cleland LG (eds) Omega-6/omega-3 Essential fatty acid ratio: the scientific evidence. World Review of Nutrition and Dietetics, vol 92. Karger, Basel, pp 1-22

Simopoulos AP (1991) Omega-3 fatty acids in health and disease and in grwoth and development. American Journal of Clinical Nutrition 54:438-463

Sinclair A, Crawford M (1972) The accumulation of arachidonate and docosahexaenoate in the developing rat brain. Journal of Neurochemistry 19:1753-1758

Smuts C, Tichelaar H, van Jaarsveld P, Badenhorst C, Kruger M, Laubscher R, Mansvelt E, Benade A (1995) The effect of iron fortification on the fatty acid composition of plasma and erythrocyte membranes in primary school children with and without iron deficiency. Prostaglandins, Leukotrienes and Essential Fatty Acids 52:59-67

Sokol R (1988) Vitamin E deficiency and neurologic disease. Annual Review of Nutrition 8:351-373

Soriguer F, Serns S, Valverde E, Hernando J, Martin-Reyes A, Soriguer M, Pareja A, Tinahones F, Esteva I (1997) Lipid, protein, and calorie content of different atlantic and mediterranean fish, shellfish, and molluscs commonly eaten in the south of spain. European Journal of Epidemiology 13:451-463

Sprecher H, Luthria DL, Mohammed B, Baykousheva SP (1995) Reevaluation of the pathways for the biosynthesis of polyunsaturated fatty acids. Journal of Lipid Research 36:2471-2477

Strittmatter P, Spatz L, Corcoran D, Rogers M, Setlow B, Redline R (1974) Purification and properties of rat liver microsomal stearyl coenzyme A desaturase. Proceedings of the National Academy of Sciences of the United States of America 71:4565-4569

Szitanyi P, Koletzko B, Mydlilova A, Demmelmair H (1999) Metabolism of 13C-Labeled Linoleic acid in newborn infants during the first week of life. Pediatric Research 45:669-673

Tinoco J (1982) Dietary requirements and functions of a-linolenic acid in animals. Progress in Lipid Research 21:1-45

T-W-Fiennes R, Sinclair A, Crawford M (1973) Essential fatty acid studies in primates linolenic acid requirements of capuchins. Journal of Medical Primatology 2:155-169

U.S. Department of Agriculture ARS (1998) USDA Nutrient Database for Standard Reference, Release 12. Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp.

U.S. Food and Drug Administration CfFSaAN (2002) Gras Notices Received in 2002. Docosahexaenoic acid-rich oil from tuna (DHA-rich tuna oil) and arachidonic acid-rich oil from Mortierella alpina (AA-rich fungal oil). http://www.cfsan.fda.gov/~rdb/opa-gn02.html, vol 2005

Uauy R, Treen M, Hoffman DR (1989) Essential fatty acid metabolism and requirements during development. Seminars in Perinatology 13:118-130

Vermunt S, Mensink R, Simonis A, Hornstra G (1999) Effects of age and dietary n-3 fatty acids on the metabolism of [13C]-alpha linolenic acid. Lipids 34:S127

Vermunt S, Mensink R, Simonis M, Hornstra G (2000) Effects of dietary alpha-linolenic acid on the conversion and oxidation of 13C-alpha linolenic acid. Lipids 35:137-142

Voss A, Reinhard M, Sankarappa S, Sprecher H (1991) The metabolism of 7, 10, 13, 16, 19 docosapentaenoic acid to 4, 7, 10, 13, 16, 19 docosahexaenoic acid in rat liver is independent of a 4-desaturase. The Journal of Biological Chemistry 266:19995-20000

Wang N, Anderston RE (1993) Synthesis of docosahexaenoic acid by retina and retinal pigment epithelium. Biochemistry 32:13703-13709

Werkman SH, Carlson SE (1996) A Randomized Trial of Visual Attention of Preterm Infants Fed Docosahexaenoic Acid Until Nine Months. Lipids 31:91-97

Yamamto N, Hashimoto A, Takemoto Y, Okuyama H, Nomura M, Kitajima R, Togashi T, Tamai Y (1988) Effect of dietary alpha-linolenate/linoleate balance on lipid compositions and learning ability of rates. II. Discrimination process, extinction process, and glycolipid compositions. Journal of Lipid Research 29:1013-1021

Ylonen K, Virtanen S, Ala-Venna E, L R (1996) Composition of diet in relation to fat intake of children aged 1-7 years. Journal of Human Nutrition and Dietetics 9:207-218

Chapter 2- Research Plan

2.1 Rationale

Arachidonic acid (AA) and docosahexaenoic acid (DHA) are important in infant growth and development and can be found in the membranes of the retina and the brain. Infants fed AA and DHA show improvement in visual and brain development. The grey matter of the brain, where DHA accumulates in large amounts during growth and development, continuously increases from birth through 5 years of age. Synaptogenesis, a period of growth spurts in the brain, peaks at eight months and reaches adult values by 11 years of age. Although the rate of growth slows, the brain and eye continue to develop throughout childhood. There is little research investigating the intake of supplementation of with AA and DHA beyond 2 years of age.

It is also important to investigate levels of AA and DHA in the child's diet, as low levels of these long chain polyunsaturated fatty acids could have a negative impact on retinal and neuronal development in children. Insufficiency in nutrients such as vitamin A, vitamin E, and iron may also have a negative impact on normal growth and development thus, it is important to assess the levels of AA, DHA, vitamin A, vitamin E, and iron in the diets of children.

2.2 Research Objectives

1. To determine the dietary intakes of AA, DHA, vitamin A, vitamin E, and iron in children living in central Alberta, who geographically are not close to a marine environment and would not be expected to have high intakes of fish or seafood.

2. To compare dietary intakes of AA and DHA of the children studied with AA and DHA intakes of infants fed human breast milk and infant formulas containing AA and DHA.
3. To supplement children identified to consume low intakes of DHA, with AA and DHA to determine if supplementation will increase essential fatty acid status and improve visual perception in children for a period of 7 months.

2.3 Hypotheses

It is hypothesized that:

1. Many of the children studied in central Alberta will consume diets low in DHA.
2. Supplementing children identified to consume low intakes of DHA, with AA and DHA will lead to an increase in AA and DHA in red blood cell phospholipids and plasma phospholipids, and an improvement of visual perception compared to children who are not supplemented with AA and DHA

2.4 Chapter Format

Chapter 3: Tests hypothesis 1 and fulfills objective 1 and 2.

This chapter assesses the nutrient intakes of DHA, AA, vitamin A, vitamin E, and iron in the current diets of 4-7 year old children.

Chapter 4: Tests hypothesis 3 and fulfills objective 3.

This chapter investigates the supplementation of DHA and AA in children 5-7 years of age and determines the effect on essential fatty acid status and visual perception.

Chapter 5 provides a general summary, overall conclusions and future research.

Chapter 3- Assessment of Docosahexaenoic acid, Arachidonic acid, Vitamin A, Vitamin E, and Iron Nutrient Intakes in 4-7 year old Children

3.1 Introduction

Long chain polyunsaturated fatty acids (LCP), specifically arachidonic acid (20:4 n-6, AA) and docosahexaenoic acid (22:6 n-3, DHA), are important in infant growth and development (Clandinin et al. 1981). These fatty acids are major components of membranes of the brain and retina (Sastry 1985; Tinoco 1982). During infant development, there is rapid accretion of AA and DHA during the last trimester of gestation (Clandinin et al. 1980). Infants born prematurely are deprived of some of this *in utero* accretion of AA and DHA unless they are fed AA and DHA (Clandinin et al. 1982). When preterm infants are supplemented with DHA, there is improvement in visual acuity (Birch et al. 1992; Carlson et al. 1993) and speed of information processing (Carlson and Werkman 1996; Werkman and Carlson 1996) was observed. In a recent double-blind multi-centre study, feeding preterm infants (n=361) formula containing AA and DHA for 118 weeks resulted in enhanced growth and higher Bayley mental and psychomotor development scores than control infants that were provided with unsupplemented formulas (Clandinin et al. 2005). In term infants, AA and DHA have also been shown to improve vision (Birch et al. 2002; Birch et al. 1998; Hoffman et al. 2003), and brain development (Agostoni et al. 1995; Birch et al. 2000; Birch et al. 2002).

The grey matter of the brain, where DHA accumulates in large amounts during growth and development (Sastry 1985; Sinclair and Crawford 1972), continuously increases from birth through 5 years of age (Levitt 2003). Although the rate of growth

slows, the brain and eye continue to develop throughout childhood (Chugani 1998; Dobbing 1972; Oyster 1999), therefore low levels of AA and DHA availability may have a negative impact on the retinal and neuronal cellular development in children during the early years of life. N-3 and n-6 fatty acids are necessary for development in both preterm and term infants and the nutritional importance of these fatty acids may extend into childhood.

Vitamin A, vitamin E, and iron are also important in normal development and growth. Vitamin A is vital in the visual process of the retina (Basu and Dickerson 1996), and α-tocopherol, the active form of vitamin E, is an important antioxidant that prevents peroxidation of polyunsaturated fatty acids (Dreyfus and Geel 1981). Prolonged deficit of vitamin E can result in neurological dysfunction (Sokol 1988). Iron deficiency during key developmental stages has a negative impact on long-term development and behavior in children (Grantham-McGregor and Ani 1999).

Dietary sources of AA and DHA include fish, shellfish, liver, poultry, eggs, beef and pork (U.S. Department of Agriculture 1998). Higher levels of DHA are found in fatty fish such as mackerel, salmon, and herring.

Geographically children in this study are not living close to a marine environment and would not be expected to have high intakes of fish or seafood. It was hypothesized that the diets of children studied contain low intakes of DHA. The purpose of this study was to determine the intake of AA, DHA, vitamin A, vitamin E, and iron in the current diets of children between 4-7 years of age using a 3-day food record. Relationship between income level and gender on DHA and AA intake was also determined.

3.2 Methods

3.2.1 Subjects

The Human Ethics Review Committee of the Faculty of Agriculture, Forestry and Home Economics, University of Alberta, Canada approved the study protocol. Principals and teachers in 8 primary schools in central Alberta agreed to participate. Information pamphlets were given to teachers of children in kindergarten and grade 1 to take home to their parents. No financial incentives were used to encourage participation. Inclusion criteria included male and female children (including siblings) between the ages of 4-7 from both low and higher income families, children with no metabolic abnormalities (e.g. diabetes), written consent from parents on behalf of their child, and at least one parent committed to attending the meetings with the researcher. Children with any chronic illness other than asthma or those with acute illness at the time of the study were not recruited. From May 2001- June 2002, a total of 91 subjects enrolled in the study. Age, gender, medical information, and parental income was recorded. Low- income cut off for a family of 3-4 persons living in this region was defined as those with total family income of less than $25 000 before taxes (Income Statistics Division 2004). Families with higher incomes were defined as those with a total family income greater than $40 000 before taxes. Children received a storybook as a token of appreciation after completion of the study. Parents were also given a copy of Canada's Food Guide to Healthy eating and were given the opportunity to ask the dietitian questions regarding the diet of their child.

3.2.2 Dietary Assessment

An initial visit was arranged with the parents where the dietitian outlined the project, reviewed participant responsibilities, obtained informed written consent (parents on behalf of their child), subject information, and medical history of the child. Parents were instructed to document all food and drink consumed by their child on a daily basis for a period of 3 consecutive days (two weekdays and one weekend day). Food records were analyzed using the USDA Nutrient Database for Standard Reference, Release 12 (U.S. Department of Agriculture 1998) in Food Processor 11, version 7.30 (1999, Esha Research, Salem Oregon). AA and DHA values for specific local foods of uncertain fat composition were determined by laboratory analysis (for example artic char, chicken nuggets, fish sticks, fish rings) and entered into the nutrient database. The quality and quantity of fat (particularly AA and DHA), as well as iron, vitamin A, and vitamin E consumed by the children was determined. To maintain consistent data analysis, all food records were manually checked and coded by one dietitian who also performed the diet history interview.

3.2.3 Fatty Acid Analysis of Specific Foods

Fat from a sample of the specific food items was extracted using the modified Folch procedure (1957). Using thin layer chromatography (TLC), total lipids were separated as described in Clandinin et al. (1997), samples were methylated (Morrison and Smith 1964) using 14% (wt/wt) boron trifluoride in methanol and C17:0 was added as an internal standard. Using gas-lipid chromatography (GLC), fatty-acid methyl esters were separated and identified (Clandinin et al. 1997). Fatty acid content was calculated from the internal standard added.

3.2.4 Statistical Analysis

Data was analyzed using the Statistical Analysis System (SAS) for Windows (SAS Institute Version 8.2, Cary NC, USA). Variables are expressed as mean± standard deviation. Mean and standard deviation was calculated for nutrient intakes measured by the 3-day food records for all subjects. One-way analysis of variance was used to examine differences between gender or between income groups for nutrient intakes (data not displayed). $P < 0.05$ was considered significant. Nutrient intake values were rounded to two significant figures. Energy, carbohydrate, vitamin A, phosphorus, potassium, and sodium intake was rounded to three significant figures.

3.3 Results

3.3.1 Subjects

A total of 91 subjects enrolled in the study, of which 78 subjects (M=39, F=39) completed. The mean age of subjects was 5.8±0.8 years (range 4.1-7.9 years). Ten families (12.8%) were in the low income group, 60 families (76.9%) have incomes >$40 000, and 8 families (10.3%) had incomes between >$25 000 and <$40 000.

3.3.2 Dietary Assessment

Macronutrient, Vitamin and Mineral Intake

The daily nutrient intake was estimated from 3-day food records from all children (Table 3-1). Macronutrient (protein, carbohydrate, and fat) intake met the acceptable macronutrient distribution ranges outlined by the Institute of Medicine (2002). Vitamin A, vitamin E, and iron intakes met the estimated average requirements (EAR) for the

children in the study, therefore, none of the children were at risk for inadequate intakes of these nutrients. In 76 of 78 subjects, fiber intakes (13±4.5 g/day) in this age group was lower than the recommended adequate intake (AI) for 4-8 year olds (25 g/day) suggested by the Institute of Medicine (2002). This was not surprising as fibre intakes in the present study were consistent with those found in the Nationwide Food Consumption Surveys in children in the US, who reported that children 2-5 years and 6-11 years had intakes of 12 g/day and 14 g/day respectively (Saldanha 1995). Fibre intakes in the children studied met fibre recommendations suggested in the US: age (years) + 5 g/day of fibre (Dwyer 1995; Williams et al. 1995).

Table 3.1 Average Daily Nutrient Intake for Children Aged 4-7 Assessed by 3-day Food Records

Nutrients per day	All subjects (n=78) X ±(SD)
Calories (kcal)	1760(440)
Protein (g)	61(17)
% of total kcal	14(2.4)
Protein (g)/ 1000 kcal	35(6.0)
Carbohydrate (g)	245(71)
% of total kcal	56(6.0)
Carbohydrate (g)/ 1000 kcal	139(15)
Fat (g)	63(17)
% of total kcal	33(4.5)
Fat (g)/ 1000 kcal	36(5.0)
Saturated fat (g)	23(7.9)
% of total kcal	12(2.4)
Monosaturated fat (g)	22(6.1)
% of total kcal	12(2.3)
Polyunsaturated fat (g)	9.8(3.6)
% of total kcal	5.1(1.6)
Fiber (g)	13(4.5)
Cholesterol (mg)	190(86)

Average intake of vitamins and minerals (Table 3-2) met the recommended EAR and AI for this age group (Institute of Medicine 1997; 1998; 2000; 2001; 2004). In 64 of 78 subjects, linoleic acid (LA) intake was lower in study subjects (7.4±3.3 g/day) compared to the AI for LA (10g/day) for those children 4-8 years of age (Table 3-3) (Institute of Medicine 2002). In 56 of 78 subjects, ALA (linolenic acid) intake was also lower (0.71±0.5 g/day) compared to the AI for ALA (0.9 g/day) for children 4-8 years of age (Institute of Medicine 2002). The omega 6 (g) to omega 3 (g) ratio was 10:1.

Table 3.2 Average Daily Vitamin and Mineral Intake for Children Aged 4-7 Assessed by 3-day Food Records

Vitamin and minerals per day	All subjects (n=78) X ± (SD)
Vitamin A (IU)	7080(3990)
Vitamin A (RE)	1290(670)
Thiamin B1 (mg)	1.7(0.7)
Riboflavin B2 (mg)	2.1(0.8)
Niacin B3 (mg)	19(7.4)
Niacin B3-NE (mg)	25(7.2)
Vitamin B6 (mg)	1.7(0.7)
Vitamin B12 (mcg)	5.4(11)
Vitamin C (mg)	150(140)
Vitamin D (IU)	290(170)
Vitamin D (mcg)	5(2.8)
Vitamin E (mg)	9.5(5.9)
Folate (mcg)	280(140)
Pantothenic acid (mg)	4.6(3.0)
Calcium (mg)	900(360)
Copper (mg)	0.94(0.5)
Iron (mg)	12(3.8)
Magnesium (mg)	200(77)
Manganese (mg)	2.7(4.0)
Phosphorus (mg)	1030(370)
Selium (mcg)	67(21.1)
Sodium (mg)	2580(822)
Zinc (mg)	10.5(18.8)

Table 3.3 Average Daily Fatty Acid Intake for Children Aged 4-7 Assessed by 3-day Food Records

Fatty acid intakes per day	All subjects (n=78) X ± (SD)
Palmitic acid 16:0 (g)	9.5(4.0)
Stearic acid 18:0 (g)	4.7(1.9)
Oleic acid 18:1 (g)	18(5.9)
Linoleic acid 18:2 (g)	7.4(3.3)
Linolenic acid 18:3 (g)	0.71(0.5)
Arachidonic acid 20:4 (mg)	57(35)
Eicosapentaenoic acid 20:5 (mg)	17(36)
Docosahexaenoic acid 22:6 (mg)	37(63)
Omega 3 (g)	0.75(0.5)
Omega 6 (g)	7.4(3.3)

AA and DHA Intake

Mean AA and DHA intake was 57±35 mg/day and 37±63 mg/day respectively. The distribution of AA intake was extensive, and spread out compared to that observed for DHA (Figure 3-1). Approximately 74% of the subjects (58 of 78) had DHA intakes ≤ 30 mg/day (Figure 3-2). Median intake for AA and DHA was 51 mg/day and 17 mg/day respectively. AA and DHA intake calculated as an average of the 3-day intake ranged from 1.2-180 mg/day and 0-350 mg/day respectively. AA and DHA intake ranged from 0-380 mg/day and 0-991 mg/day respectively when intakes were calculated per day, which show a large intra (within person) and inter (between person) variation.

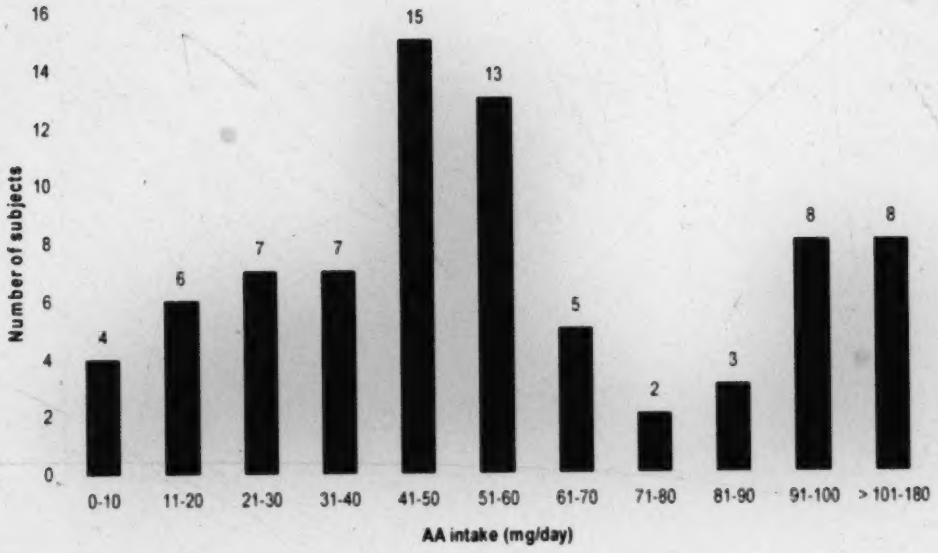


Figure 3.1 Distribution of AA Intake (mg/day) Assessed by 3-day Food Records for Children Aged 4-7

Caption: AA intake (mg/day) was expressed in 10 mg/day increments and the number of children in each 10 mg/day increment is displayed in the bar graph.

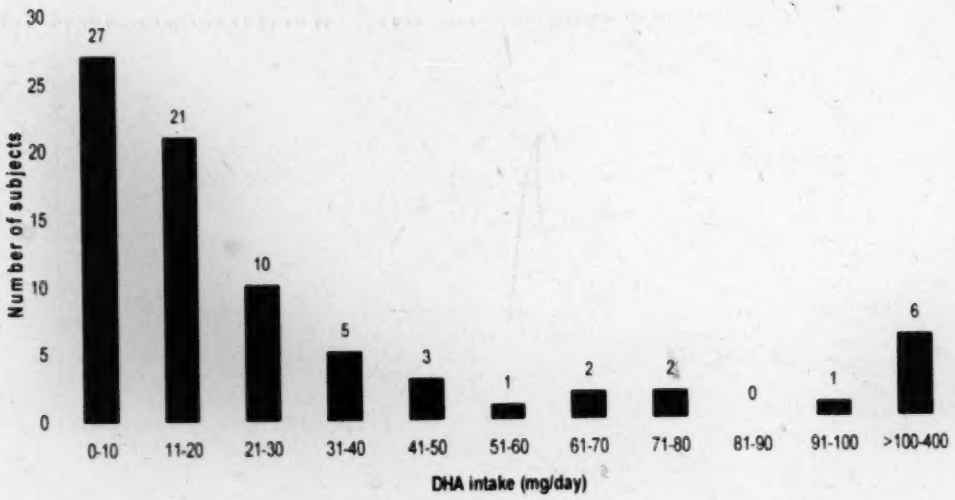


Figure 3.2 Distribution of DHA Intake (mg/day) Assessed by 3-day Food Records for Children Aged 4-7

Caption: DHA intake (mg/day) was expressed in 10 mg/day increments and the number of children in each 10 mg/day increment is displayed in the bar graph. Approximately 74% of subjects (58 of 78) had DHA intakes ≤30 mg/day.

Gender and Income Comparison.

Nutrient intake (macronutrient, vitamin, minerals, and fatty acids) was not significantly different between males and females. When nutrient intakes were determined for the low income group compared with higher income group, all nutrients met the recommended EAR and AI and were not significantly different between the two groups except for AA ($P<0.05$). AA intake was significantly higher in the lower income group (78.7±33.9 g/day) than in the higher income group (53.3±33.6 g/day), which was likely due to a higher consumption of eggs.

3.4 Discussion

In the present study, children, 4-7 years of age, met the EAR levels suggested by the Institute of Medicine for vitamin A, vitamin E, and iron, which indicates that the children were at low risk of having inadequacies of these specific nutrients (2000; 2001). Mean intake of AA and DHA in children was estimated to be 57 mg/day and 37 mg/day using a 3-day food record. This present study found that AA intake was higher in families having a low income (<$25 000) compared to families having an income >$40 000, whereas DHA intake was not different between the two income groups.

3.4.1 Macronutrient, Vitamin and Mineral Intakes

Children achieved nutrient intakes for macronutrients, vitamins and minerals recommended for the acceptable macronutrient distribution ranges, EAR and AI by the Institute of Medicine (1997; 1998; 2000; 2001; 2002; 2004) (Table 3-1 and Table 3-2). In particular, vitamin A, vitamin E, and iron intakes met the EAR levels, which indicates

that the children studied were not at risk of having inadequacies of these nutrients due to dietary intake.

3.4.2 Food Assessment Method used to Assess AA and DHA Intakes

The food assessment method used to assess AA and DHA intake may be another reason why wide variability of AA and DHA intake was observed. In the present study, diet was assessed using a 3-day unweighed food record. Use a 7-day weighted food record maybe considered a more precise method available for estimating usual food and/or nutrient intakes of individuals, so long as respondents do not change their usual eating pattern (Gibson 1990). This method was considered to be too much of a burden to parents participating in the present study and would have resulted in problems with compliance completion rates. Dierks and Morse (1965), estimated nutrient intakes in preschool children using 3-day food records and report a 95% completion rate. The present study had a completion rate of 86%. The method used in the current study is similar to the method used by Allison et al. (1999) and Jonnalagadda et al. (1995) where food intake was collected over a three day period (one 24 hour recall, and 2-day food record). Although a 7-day weighted food record would be beneficial to use in the studies conducted by Allison et al. (1999) and Jonnalagadda et al. (1995), it would not have been feasible due to the number of participants in these studies. A 24-hour recall method was used by Meyer et al. (2003) however a single day is not representative of the usual fat consumption of an individual (Block 1982; Pekkarinen 1970). Food frequency questionnaires provide an inferior quantitative estimate of intake as this method does not usually define information on a specific food items consumed, exact portion sizes

consumed, food preparation methods, brand name and packaging information (Allison et al. 1999; Briefel et al. 1992; Sempos 1992). Innis et al. (2004) used a food frequency questionnaire, and considered this limitation and collected data on specific foods (e.g. frequency food was eaten, portion sizes, brand name or place of purchase, method of preparation, use of fat-reduced foods, types of margarines, shortenings, and other fats and oils), therefore, AA and DHA nutrient estimates are likely to be representative of the preschool children studied. Raper et al.(1992) and Kris-Etherton (2000), used per capita food use data or per capita disappearance data to estimate DHA intakes, however, this method overstates actual consumption as it does not account for spoilage and waste, and is not a direct measure of the quantity of food actually consumed (Economic Research Service 2004).

3.4.3 Intakes of AA and DHA

Few studies report AA and DHA intake in either children or adults. Of these studies, the data reported varies. For instance, in Australian children (aged 4-7 years, n=799), AA and DHA intake was 22 mg/day and 47 mg/day respectively, thus AA intakes from the present study was slightly higher (57 mg/day), whereas the DHA intakes were slightly lower (37 mg/day) (Table 3-3) than the intakes estimated from the National Nutritional Survey (Meyer et al. 2003). The same study also investigated the AA and DHA intake in Australian adults (≥19 years of age, n=10 851) and found that AA intakes were similar to the AA intakes found in the present study, however DHA intakes were 69 mg/day higher than the DHA intakes in the present study. Meyer et al. (2003) also reported the median intakes of AA and DHA in adults to be 24 mg/day and 15 mg/day

respectively. Median levels of AA from Meyer et al. (2003) was half the value from the present study, while their DHA median value was consistent to the findings in the present study. However, it would have been beneficial to compare median values for children in the targeted age group.

Other research studies have reported higher AA and DHA intakes than those estimated in the present study. Jonnalagadda et al. (1995) estimated fatty acid intake of children (aged 6-11, n=992) determined from data using 1987-1988 US Nationwide Food Consumption Survey and found that intake of 18:4+AA was 100±0 mg/day and for 20:5+DHA was 100±0 mg/day. Similar results were found by Allison et al. (1999), who estimated fatty acid intake from the 1989-1991 US Continuing Survey of Food Intakes of Individuals (n=11 258). Fatty acid intake for those individuals 3 years of age and older was 100±10 mg/day for 18:4+AA and 100±10 mg/day for 20:5+DHA. In a study done by Innis et al.(2004), Canadian children 3-5 years of age, had higher AA (226±17 mg/day) and DHA (96±14 mg/day) intakes than in the present study. Raper et al. (1992) investigated annual per capita food use data in the US food supply and found that the DHA levels have increased from 69 to 78 mg/capita/day between 1935-1939 and 1985 and was the result of increased use of canned tuna, gamefish, and poultry. Using per capita disappearance data of fish, Kris-Etherton (2000) estimated DHA intakes in the United States as 0.25 g/day and worldwide as 0.23 g/day.

The geographic location where nutrient assessments were completed is one of the reasons why wide variability of AA and DHA intake was observed. Many of the nutrient assessments were national surveys (Allison et al. 1999; Jonnalagadda et al. 1995; Meyer et al. 2003) which provide the mean intakes of AA and DHA for all subjects in the study.

Although it is important to have an idea of the intakes of AA and DHA consumed by a population, it may not indicate areas that may have lower intakes of these fatty acids. In the study by Innis et al.(2004), DHA intake was expected be to higher than the present study, as the preschool children studied were from the Vancouver Costal Health Authority Region, British Columbia, Canada, an area where seafood is readily available. It is important to compare the median values of AA and DHA, as it represents the middle of a distribution and maybe more informative. In the present study, the median and mean intake of AA are similar, however, the median DHA intake is approximately half of the mean intake for DHA.

3.4.4 Inter and Intra Variation in AA and DHA Intakes

The distribution (Figure 3-1 and Figure 3-2) and range of AA and DHA intake (over the 3-days and per day basis) is evidence of inter and intra variation of AA and DHA intakes in the children studied. Since dietary sources rich in AA and DHA (e.g. fatty fish and seafood), are not consumed on a daily basis, it was expected that the levels would vary widely day to day. Low dietary levels of AA and DHA observed in the present study, accompanied by the varied day to day intakes of these fatty acids are of concern because these fatty acids are essential for normal infant growth and development (Clandinin et al. 2005). The rate of LCP synthesis from precursors has not been explored in children. Since the brain and eye continue to develop throughout childhood (Chugani 1998; Dobbing 1972; Oyster 1999), it is likely that AA and DHA intake is important in children.

3.4.5 AA and DHA Intakes Compared to Infant Values

AA and DHA intake in the children studied (Table 3-3) is lower than the intake of AA and DHA in infants consuming mother's milk or formulas containing AA and DHA. To estimate AA and DHA (mg/kg body weight/day) intake in infants, the following assumptions can be made: energy requirement of the infant is 100-120 kcal/kg body weight/day, human milk provides ~55% of calories from fat. Using the AA and DHA levels in human milk reported earlier (Clandinin et al. 1997), infant intake level of AA range from 33-40 mg/kg body weight/day and DHA intake range from 18.3-22 mg/kg body weight/day. Using the AA and DHA levels provided in infant formula (for example: Enfamil A+ (Canadian brand) or Enfamil ® Lipil ® with Iron (US brand) (Mead-Johnson Nutritionals, Evansville, IN)), with assumed energy requirements of an infant is 100-120 kcal/kg, infant intake level of AA ranged from 34-41.2 mg/kg body weight/day and DHA level ranged from 17.1-20.6 mg/kg body weight/day.

In the present study, if the assumed weight of the child, 4-7 years of age is 25 kg, mean AA and DHA intake observed provides approximately 2.3 mg/kg body weight/day of AA and 1.5 mg/kg body weight/day of DHA respectively. The intakes in the present study are lower than the AA and DHA intake observed in Innis et al. (2004), where children 3-5 years of age (assumed weight of a child is 16 kg), mean AA and DHA intake was 14 mg/kg body weight/day and 6.0 mg/kg/body weight day respectively.

Many of the children in the present study have low intake of LA and ALA, that is 82% and 72% respectively did not meet the AI suggested for LA and ALA (Institute of Medicine 2002). LA and ALA are essential fatty acids, and are necessary for the desaturation and elongation to their metabolites, AA and DHA. The amount of AA and

DHA synthesized from LA and ALA, along with low dietary intake of AA and DHA, may not be sufficient to support the AA and DHA levels necessary for vision and brain development in the growing child.

The current level of AA and DHA provided by the diet of the children in the present study is approximately 1/15 the amount of AA and 1/12 the amount of DHA that is available to infants fed human milk or formulas containing AA and DHA. An increased intake of AA and DHA may be necessary to support retinal and neuronal cellular development since the brain and the eye continue to develop throughout childhood.

3.4.6 Income Comparison

The present study is the first study that compared AA and DHA nutrient intake in children to parental income status. The data in this study showed that AA intake was significantly higher in the low income group, than the higher income group, which was due to the increased consumption of eggs. DHA intakes did not differ significantly between income groups. The findings in the present study also showed that DHA intake was not different between low and higher income groups.

3.5 Conclusion

The present study showed that diets of Canadian children aged 4-7, who geographically do not live near a marine environment have dietary levels of AA and DHA that are low compared to the few other studies that have previously investigated dietary fat intake in children (Allison et al. 1999; Innis et al. 2004; Jonnalagadda et al.

1995; Meyer et al. 2003). The current level of AA and DHA provided by the diet of the children is approximately 1/15 the amount of AA and 1/12 the amount of DHA that is available to infants fed human milk or formulas containing AA and DHA. An increased intake of AA and DHA may be necessary to support retinal and neuronal cellular development since the brain and the eye continue to develop throughout childhood.

3.6 References

Agostoni C, Trojan S, Bellu R, Riva E, Giovannini M (1995) Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: the role of long-chain polyunsaturated fatty acids. Pediatric Research 38:262-266

Allison DB, Egan K, Barraj LM, Caughman C, Infante M, Heimbach JT (1999) Estimated intakes of trans fatty and other fatty acids in the US population. Journal of the American Dietetic Association 99:166-174

Basu T, Dickerson J (1996) Vitamins in human health and disease. Cab International, Wallingford, Oxon

Birch EE, Birch DG, Hoffman DR, Uauy R (1992) Dietary essential fatty acid supply and visual acuity development. Investigative Ophthalmology and Visual Science 33:3242-3253

Birch EE, Garfield S, Hoffman DR, Uauy R, Birch DG (2000) A randomized controlled trial of early dietary supply of long-chain polyunsaturated fatty acids and mental development in term infants. Developmental Medicine and Child Neurology 42

Birch EE, Hoffman DR, Castaneda YS, Fawcett SL, Birch DG, Uauy RD (2002) A randomized controlled trial of long-chain polyunsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age. American Journal of Clinical Nutrition 75:570-80

Birch EE, Hoffman DR, Uauy R, Birch DG, Prestidge C (1998) Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. Pediatric Research 44:201-209

Block G (1982) A review of validations of dietary assessment methods. American Journal of Epidemiology 115:492-505

Briefel R, Flegal K, Winn D, Loria C, Johnson C, Sempos CT (1992) Assessing the nation's diet: limitations of the food frequency questionnaire. Journal of the American Dietetic Association 92:959-962

Carlson SE, Werkman SH (1996) A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until two months. Lipids 31:85-90

Carlson SE, Werkman SH, Rhodes P, Tolley E (1993) Visual acuity development in healthy preterm infants: effect of marine-oil supplementation. American Journal of Clinical Nutrition 58:35-42

Chugani HT (1998) A critical period of brain development: studies of cerebral glucose utilization with PET. Preventive Medicine 27:184-188

Clandinin M, Chappell J, Heim T (1982) Do low weight infants require nutrition with chain elongation-desaturation products of essential fatty acids? Progress in Lipid Research 20:901-904

Clandinin M, Chappell J, Heim T, Swyer P, Chance G (1981) Fatty acid utililzation in perinatal de novo synthesis. Early Human Development 5:355-366

Clandinin M, Chappell J, Leong S, Heim T, Swyer P, Chance G (1980) Intraunterine fatty acid accretion rates in human brain: implications for fatty acid requirements. Early Human Development 4:121-129

Clandinin M, Van Aerde J, Parrott A, Field C, Euler A, Lien E (1997) Assessment of the efficacious dose of arachidonic and docosahexaenoic acids in preterm infant

formulas: fatty acid composition of erythrocyte membrane lipids. Pediatric Research 42:819-825

Clandinin MT, Van Aerde JE, Merkel KL, Harris CL, Springer MA, Hansen JW, Diersen-Schade D (2005) Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. The Journal of Pediatrics

Dierks EC, Morse LM (1965) Food habits and nutrient intakes of preschool children. Journal of the American Dietetic Association 47:292-296

Dobbing J (1972) Vulnerable periods in of brain development. In: K E, Knight J (eds) Lipids, Malnutrition and the developing brain. Elsevier, Amsterdam, pp 9-20

Dreyfus PM, Geel SM (1981) Chapter 33: Vitamin and Nutritional Deficiencies. In: Siegel GJ, Albers RW, Agranoff BW, Katzman R (eds) Basic Neurochemistry. Little, Brown and Company, Boston

Dwyer JT (1995) Dietary fiber for children: how much? Pediatrics 96:1019-1022

Economic Research Service USDoA (2004) Food consumption: food supply and use. http://www.ers.usda.gov/Data/FoodConsumption/, vol 2004

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. The Journal of Biological Chemistry 226:497-509

Gibson R (1990) Principles of Nutritional Assessment. Oxford University Press, New York

Grantham-McGregor S, Ani C (1999) The role of micronutrients in psychomotor and cognitive development. British Medical Bulletin 55:511-527

Hoffman DR, Birch EE, Castaneda YS, Fawcett SL, Wheaton DH, Birch DG, Uauy R (2003) Visual function in breast-fed term infants weaned to formula with or without long-chain polyunsaturates at 4 to 6 months: a randomized clinical trial. Journal of Pediatrics 142:669-677

Income Statistics Division (2004) Low income cutoffs from 1994-2003 and low income measure from 1992-2001. Statistics Canada Catalogue no. 75F002MIE- No. 002:1-38

Innis SM, Vaghri Z, King DJ (2004) n-6 Docosapentaenoic acid is not a predictor of low docosahexaenoic acid status in Canadian preschool children. American Journal of Clinical Nutrition 80:768-73

Institute of Medicine (1997) Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. National Academy Press, Washington, DC

Institute of Medicine (1998) Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline. National Academy Press, Washington, DC

Institute of Medicine (2000) Dietary Reference Intakes for vitamin C, vitamin E, selenium and carotenoids. National Academy Press, Washington, DC

Institute of Medicine (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press, Washington, DC

Institute of Medicine (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). National Academies Press, Washington, DC

Institute of Medicine (2004) Dietary reference intakes for water, potassium, sodium, chloride, and sulfate. National Academies Press, Washington, DC

Jonnalagadda SS, Egan KS, Heimbach JT, Harris SS, PM K-E (1995) Fatty acid consumption pattern of Americans: 1987-1988 USDA Nationwide Food consumption survey. Nutrition Research 15:1767-1781

Levitt P (2003) Structural and functional maturation of the developing primate brain. Journal of Pediatrics 143:S35-45

Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair A, Howe PR (2003) Dietary intakes and food sources of omega 6 and omega 3 polyunsaturated fatty acids. Lipids 38:391-398

Morrison WR, Smith LM (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research 5:600-608

Oyster CW (1999) The Human Eye- Structure and Function. Sinauer Associates Inc, Sunderland, Massachusetts

Pekkarinen M (1970) Methodolgy in the collection of food consumption data. World Review of Nutrition and Dietetics 12:145-171

Raper NR, Cronin FJ, Exler J (1992) Omega-3 fatty acid content of the US food supply. Journal of the American College of Nutrition 11:304-308

Saldanha LG (1995) Fiber in the diet of US children: results of national surveys. Pediatrics 96:994-997

Sastry P (1985) Lipids of nervous tissue: composition and metabolism. Progress in Lipid Research 24:66-176

Sempos CT (1992) Invited commentary: some limitations of semiquantitative food frequency questionnaires. American Journal of Epidemiology 135:1127-1132

Sinclair A, Crawford M (1972) The accumulation of arachidonate and docosahexaenoate in the developing rat brain. Journal of Neurochemistry 19:1753-1758

Sokol R (1988) Vitamin E deficiency and neurologic disease. Annual Review of Nutrition 8:351-373

Tinoco J (1982) Dietary requirements and functions of a-linolenic acid in animals. Progress in Lipid Research 21:1-45

U.S. Department of Agriculture ARS (1998) USDA Nutrient Database for Standard Reference, Release 12. Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp.

Werkman SH, Carlson SE (1996) A Randomized Trial of Visual Attention of Preterm Infants Fed Docosahexaenoic Acid Until Nine Months. Lipids 31:91-97

Williams CL, Bollella M, Wynder EL (1995) A new recommendation for dietary fiber in childhood. Pediatrics 96:985-988

Chapter 4- Consuming a nutritionally complete supplement containing AA and DHA for 7 months improves visual perception in children 5-7 years of age

4.1 Introduction

Essential fatty acids, linoleic acid (C18:2n-6, LA) and linolenic acid (C18:3n-3, ALA) are precursors of long chain polyunsaturated fatty acids (LCP), arachidonic acid (20:4n-6, AA) and docosahexaenoic acid (22:6n-3, DHA). These LCP are major components of the membranes of the brain and retina (Sastry 1985; Tinoco 1982) and play an important role in infant growth and development (Clandinin et al. 1981). During the third trimester, rapid synthesis of the brain occurs with marked accretion of AA and DHA (Clandinin et al. 1980), however, preterm infants are deprived of some of this *in utero* accretion (Clandinin et al. 1982). When preterm infants are supplemented with DHA, there is improvement in visual acuity (Birch et al. 1992; Carlson et al. 1993) and speed of information processing was observed (Carlson and Werkman 1996; Werkman and Carlson 1996). In a recent double-blind multi-centre study, feeding preterm infants (n=361) formula containing AA and DHA for 118 weeks resulted in enhanced growth and higher Bayley mental and psychomotor development scores than control infants who were provided with formulas without AA and DHA (Clandinin et al. 2005). Studies in term infants have shown an improvement in vision (Birch et al. 2002; Birch et al. 1998; Hoffman et al. 2003) and brain development (Agostoni et al. 1995) for infants given formulas containing AA and DHA.

The grey matter of the brain, where DHA accumulates in large amounts during growth and development (Innis 2003), continuously increases from birth through 5 years

of age (Levitt 2003). Although the rate of growth slows, both brain and eye development continues throughout childhood (Chugani 1998; Dobbing 1972; Oyster 1999). A child's brain weighs 90% of the adult value by age of five, and reaches the full weight of an adult brain at 10-12 years of age (Paus et al. 2001). Synaptogenesis (period of growth spurts in the brain) peaks at eight months and reaches adult values by 11 years (Garey 1984; Garey 1983; Huttenlocher et al. 1982). Studies have not investigated the role of AA and DHA in functions of the developmental process in children beyond 2 years of age.

Dietary sources of AA and DHA are found mainly in fish, shellfish, liver, poultry, beef, pork, and eggs (U.S. Department of Agriculture 1998). Higher DHA levels are found in fatty fish such as mackerel, herring, and salmon. Children that were geographically not close to a marine environment consumed low levels of DHA, with 74% of the children surveyed having intakes of ≤30mg/day (Lien and Clandinin unpublished). In the present study, children ages 5-7 years, who consume low intakes of DHA, were provided with either a dietary supplement containing AA and DHA or the same supplement without AA and DHA, for a period of 7 months. It is hypothesized that supplementing children for a period of 7 months with AA (20-30 mg/day) and DHA (14-21 mg/day) will lead to increase in AA and DHA in phospholipids in red blood cells and plasma phospholipid, and that increased AA and DHA intake will improve visual perception compared to children who are not supplemented with AA and DHA.

4.2 Methods

4.2.1 Subjects

The Human Research Ethics Boards of the Faculty of Agriculture, Forestry and Home Economics, and the Faculty of Medicine at the University of Alberta approved the study protocol. Healthy school aged children living in the central Alberta, Canada, who had participated in the a previous study to assess nutrient intakes of AA, DHA, vitamin A, vitamin E, and iron in children 4-7 years of age (Chapter 3), were considered for the present study. DHA intake was assessed in 91 subjects using 3-day food records (Chapter 3). From May 30, 2001 to July 15, 2002, subjects in with DHA intakes of less than 78.0 mg/day were contacted and recruited for the present study (n=37). A food frequency questionnaire completed in the initial assessment and used to verify low DHA intakes reported in the 3-day food records.

4.2.2 Inclusion and Exclusion Criteria

Healthy male and female children including siblings between the ages of 4-7, who consumed a level of DHA intake less than 78.0 mg/day were recruited (Chapter 3). Informed written consent from parents on behalf of their child was obtained. Exclusion criteria included the presence of a chronic illness (other than asthma) or metabolic disease, milk aversion or intolerance to milk proteins or lactose, consumption of fish oil supplements for more than 4 weeks within the past 6 months, and participation in any other clinical study.

4.2.3 Study Design

The study design consisted of a controlled, randomized, double blind study. Age, sex, subject information, and a medical history was recorded. All routine immunizations, vitamins and mineral supplements consumed, and use of prescription medications or therapies were recorded.

To ensure normal vision all children completed an ophthalmic examination at the Ophthalmology Clinic at the University of Alberta at baseline (0 months) and at the completion of the study (7 months). Weight and height, neurological assessment, 4-day food records, and food frequency questionnaires was conducted at 0 and 7 months. Food records were also collected during the 3rd month. Blood samples were collected by fingerprick by a phlebotomist at 0 months and a venous blood sample was collected at 7 months.

Parents were given a supply of 100 sachets of the assigned supplement. Compliance was evaluated based on planning a schedule in a monthly calendar provided to the parents. One sticker per sachet was placed in a calendar when consumed. Thus normally two to three stickers per day would be visible on the calendar. Parents also completed a health diary on a monthly basis. Parents were instructed to provide one sachet per day to their child and then progress to 2-3 sachets per day. Parents could contact the study coordinator at any time.

Monthly visits were arranged with the parent and study coordinator. At this visit sachets remaining were counted, parents were provided with more sachets for the following month, calendars and health diaries were collected, checked, and new calendars

and health diaries were distributed. Compliance was assessed by asking parents questions monthly such as if their child liked the formula (positive, negative, or neutral), and if their child tolerated the beverage, if their child was taking 2-3 sachets per day, if their child had regular bowel movements, and if their child had been ill during the past month. There were two highlighted dates on the monthly calendar where children were given prizes (e.g. pens, pencils, craft kits, kites, erasers).

Parents were repeatedly counseled on the importance of ensuring their child consumed two to three sachets per day. Some children were unable to meet a minimum intake of two sachets per day required. The study completion date was calculated based on the total number of sachets that would have been achieved if the child had consumed at least two sachets per day, or 420 packets (e.g. 2 sachets/day x 30 days/month x 7 months). For children who ingested only 1-1.5 packets per day, the final completion date was determined based on achieving an intake goal of 420 packets. Four subjects remained in the study for up to 8 weeks more than the estimated 7 months, one subject up to 12 weeks more, and one subject that remained in the study for up to 16 weeks more than the estimated 7 months. The study endpoint will be referred to as 7 months throughout this paper.

4.2.4 Dietary Formula

Subjects received one of two nutritionally complete formulas. The formula provided was Promil™ a cow's milk-based vanilla flavoured nutritionally complete formula (Wyeth Nutritionals Incorporated, Philadelphia, PA). The control formula (control group) did not contain AA and DHA. The experimental supplement (LCP

group) PromilGold™ was the same product, containing AA and DHA. The fatty acid content of the control formula consisted of the following per sachet (200 mL serving): 130 mg C8:0, 122 mg C10:0, 720 mg C12:0, 324 mg C14:0, 1.58 g C16:0, 324 mg C18:0, 21.6 mg C20:0, 14.4 mg C22:0, 3.24 g total saturated fat, 7.20 mg C16:1, 2.66 g C18:1, 14.4 mg C20:1, 2.69 g total monounsaturated fat, 1.17 g C18:2, 108 mg C18:3, and 1.28 g total polyunsaturated fat (Appendix A, Table A.1). The fatty acid content of the LCP supplement consisted of the following per sachet (200 mL serving): 84.0 mg C8:0, 72.8 mg C10:0, 521 mg C12:0, 241 mg C14:0, 1.14 g C16:0, 245 mg C18:0, 16.8 mg C20:0, 11.2 mg C22:0, 5.60 mg C24:0, 2.34 g total saturated fat, 11.2 mg C16:1, 2.00 g C18:1, 11.2 mg C20:1, 2.02 g total monounsaturated fat, 1.09 g C18:2, 123 mg C18:3, 10.4 mg C20:4, 7.00 mg C22:6, and 1.23 g total polyunsaturated fat (Appendix A, Table A.1). The nutrient composition of control and LCP formula consisted of the following per sachet: 134 kcal energy, 4.40 g protein, 16.4 g carbohydrate, 13.1 g 80% lactose, 3.28 g 20% sucrose, 5.60 g fat, 500 IU vitamin A, 85.0 IU vitamin D, 2.20 IU vitamin E, 13.4 mcg vitamin K, 200 mcg vitamin B1, 300 mcg vitamin B2, 120 mcg vitamin B6, 0.400 mcg vitamin B12, 0.800 mg niacin, 16.0 mcg folic acid, 600 mcg pantothenic acid, 4.00 mcg biotin, 18.0 mg vitamin C, 20.0 mg choline, 230 mg calcium, 130 mg phosphorus, 17.0 mg magnesium, 2.40 mg iron, 1.00 mg zinc, 20.0 mcg manganese, 80.0 mcg copper, 26.0 mcg iodine, 70.0 mg sodium, 200 mg potassium, 150 mg chloride, and 2.80 mcg selenium (Appendix B, Table B.1). Both formulas were packaged as one sachet per individual serving. Parents were required to reconstitute the formula by adding approximately 180 mL of drinking water to the powdered formula in a shaker cup

supplied with lid, and to provide at least two, preferably three 200 mL servings of the formula to their child daily until the end of the study.

During the study, some children (n=8) complained about the taste of the formula. To retain subjects, alternate ways to consume the formula were developed. Parents could change the flavour by adding strawberry or chocolate syrup to the formula. Standardarized recipes were developed so that one sachet would be present in one serving. A sachet could be added to yogurt, ice cream, hot chocolate, instant pudding, frozen treats, oatmeal, muffins, brownies, cookies, and pancakes. A few parents (LCP group n=1, Control group n=2) were provided with the supplement in the form of muffins and/or cookies on a monthly basis.

4.2.5 Randomization

During the clinical study, the study site was blinded to the dietary formula consumed by each child. Formula assignments were uncoded when the study was completed. Children were randomized to receive one of the two possible dietary formulas on the day of their first ophthalmic exam. Each of the two formulas were further masked by two colour/alphabet codes, for a total of four possible assignments for each subject. A randomization schedule was developed by Wyeth Nutritionals Incorporated and provided in sealed envelopes to the study site in advance of recruitment.

4.2.6 Growth

Height and weight was measured at the start and end of the study. Weight and height was measured using a sliding weight scale/combination height rod. Height and

weight was assessed three times, the mean of the height and weight was recorded. Body mass index (BMI) was calculated based on height and weight obtained at 0 and 7 months.

4.2.7 Diet Assessment

Parents were instructed by the dietitian to document all food and drink consumed by their child on a daily basis for a period of four consecutive days (three weekdays and one weekend day). Food records were analyzed using the USDA Nutrient Database for Standard Reference, Release 12 (U.S. Department of Agriculture 1998) in Food Processor 11, version 7.30 (1999, Esha Research, Salem Oregon). AA and DHA values for some local foods were analyzed in the laboratory (e.g. artic char, chicken nuggets, fish sticks, fish rings) and entered into the nutrient database. Quality and quantity of fat (particularly AA and DHA), macronutrients, vitamins and minerals consumed by the children was determined. The 4-day food record was collected at 0, 3, and 7 months of the study. Intake from formula packets was included in the food record for the 4-day food record at 3 and 7 months. Thus for months 3 and 7, in the LCP group, the food record data has been separated into intake that contains LCP supplement and intake that excludes the LCP supplement. This allows determination of the intake of macronutrients, energy, and of AA and DHA in the LCP group from food sources other than that provided by the supplement, as well as assessment of the total amount of AA and DHA consumed from food sources and the supplement provided.

The child's usual food intake pattern was also assessed using a 49-item food frequency questionnaire collected at 0 and 7 months (See Appendix C for sample Food Frequency Questionnaire). Questions regarding the consumption of certain fish,

seafood/shellfish, liver, egg yolks, chicken, turkey or other poultry on a monthly and/or weekly basis were recorded. During diet interviews, food models were used to help parents visualize food portions consumed by their child. To maintain consistency in data analysis, the food records and food frequency questionnaires were manually checked and coded by one dietitian who also performed all diet interviews.

4.2.8 Visual Function Assessment

A complete ophthalmic exam was performed on all children to ensure normal visual status of all children before and after supplementation. It is important to determine if a change in the neurological development score was the result of changes in LCP status and not abnormal vision. Five age appropriate visual tests for visual acuity, stereo acuity, colour vision, cyclo refraction, and strabismus were conducted (See Appendix D, Figure D.1 to Figure D.4). Visual acuity (the minimum discrimination of an object at a fixed distance) was determined using the HOVT chart (Hedin et al. 1980). This chart consists of four random letters (HOVT) of different sizes, and was placed 10 feet away, while the child was required to read off the letters while one eye was covered. Left and right eyes were tested separately, and the final score of correct letters read out of 44 was recorded. The Lang test was used to measure stereo acuity (depth perception) (Lang 1983). This test consists of random dots, where an image can be found within the random dots and can only be seen if the child has stereo viewing. A yes or no response was recorded. Colour vision (the ability of the child to distinguish between different colours) was assessed using Ishihara pseudoisochromatic plates. The plates have multicolour dots which form numbers when the same hue and saturation of colour is present (Lakowski

1965). Individuals who are colour blind are unable to detect certain numbers or may see different numbers than subjects who are not colour blind. A modified test was used where only eight plates (with single digit numbers) were shown to the child, instead of using plates that had two digits. Left and right eyes were tested and a score out of 8 was recorded for each eye. Cyclo refraction (shape of the eye) was determined for each child. Prior to this test, it was necessary use dilating drops or cycloplegics to paralyze the focus mechanism of the eye to help prevent accommodation, thus allowing for accurate measurement of the sphere or curve of both the left and right eye. The prism cover test was used to determine if a child had strabismus (cross-eyed). One eye would be covered with a paddle, and if the uncovered eye shifted or moved, then a prism was used to determine the angle of movement in the eye. This was recorded at both near (reading distance) and at a distance (20 feet or 6 metres). After the ophthalmic exams, if a child needed prescription glasses, these were obtained prior to entry in the study. Two children required prescription glasses at baseline. Once prescription lenses were obtained, both children were rescheduled to complete the ophthalmic exams again before they were accepted into the study.

4.2.9 Neurological Assessment

Visual perception was assessed using the Test of Visual Perceptual Skills (non-motor)-Revised (TVPS-R) (Gardner 1996). Visual perception is the ability of the brain to comprehend and interpret, or give meaning to what the eyes see, and to express the meaning verbally or motorically (Gardner 1996). This is a standardized test developed for children 4 years through 12 years, and measures visual perceptual skills using seven subtests: visual discrimination (identification and matching of similar forms); visual

memory (immediate recall of a single form); visual spatial-relationships (identification of the correct direction of forms); visual form-constancy (identification of a form, whether it is a different size, rotated, reversed, or hidden in or among other forms); visual sequential-memory (immediate recall of a number of forms in a series); visual figure-ground (finding a form that is hidden among other forms); visual closure (determination of a whole form from incomplete parts of the form) (See Appendix E, Figure E.1). For each subtest, there are 16 items (from 0- all failed to 16- all correct). Correct responses yielded a 'raw score' that is translated into scaled scores, and into a median visual perceptual age. The psychologist contacted parents and arranged to conduct both the baseline and endpoint assessments at the child's home to ensure a comfortable environment for the child.

4.2.10 Health Diary

Parents were provided with a health diary to record when their child was ill, length of days the illness persisted, symptoms, name of disease or condition (if known), and whether any prescription medications/over the counter medication was taken throughout the study period. All vitamin and mineral supplements taken were documented, and any adverse events were recorded throughout the study period.

4.2.11 Fatty Acid Analysis

Fingerpricks (~100 uL of blood) were performed on the children at 0 months and collected into a microtainer tube containing heparin, and venous blood samples (~2.5 mL of blood) were drawn into a green top Vacutainer tube containing heparin at 7 months.

All samples were taken by a phlebotomist in an outpatient clinic and placed on ice for transport back to the laboratory for analysis.

Sterile supplies were obtained from Fisher Scientific (Edmonton, Alberta). All blood samples were immediately centrifuged at 3000 rpm for 10 minutes (Jouan CR 422 Canbera Packard, Missisauga, Ontario) at room temperature (20^0C). Plasma was drawn off from samples and stored in eppendorf tubes and saved at -70^0C for later lipid analysis. Red blood cells (RBC) were also drawn off for immediate lipid analysis.

Lipids were extracted from 50 uL of plasma (Folch et al. 1957), using a total of 2 mL choloroform methanol (2:1 vol/vol). Lipids were extracted from 50 uL of red blood cells using both the Rose and modified Folch procedure (Folch et al. 1957; Rose and Oklander 1965). Lipids from sample of local food items were extracted using the modified Folch procedure (1957). Total phospholipids, cholesteryl esters, and triacylglycerol were separated by thin layer chromatography (TLC) (Clandinin et al. 1997) and samples were methylated (Morrison and Smith 1964) using 14% (wt/wt) boron trifluoride in methanol. C17:0 was added as an internal standard. Fatty-acid methyl esters were separated by gas-liquid chromatography (GLC) (Clandinin et al. 1997), and retention times of each of the fatty acid methyl ester sample was compared with those of a fatty acid methyl ester standard (NU-CHEK PREP, Inc) containing 32 known fatty acid methyl esters. Fatty acid content was calculated from the internal standard added.

4.2.12 Sample Size

There is limited data quantifying plasma DHA levels in children thus sample size was estimated using normal, healthy control children (n=8) approximately 8 years of age

living in a similar area from a previous study (Clandinin et al. 1995). Based on the mean and standard deviation values for the DHA content in the total plasma phospholipids of control subjects at pre and post supplementation, final sample size per group for a two tailed test ($1-\beta=0.90$, $\alpha=0.05$) was 8, and sample size per group for a one tailed test ($1-\beta=0.90$, $\alpha=0.05$) was 7 (Bausell and Li 2002). In the present study, a sample size of 12 per group was considered to be adequate. A total of 37 subjects were recruited in the present study, 20 subjects in the LCP group, and 17 subjects in the control group to allow for drop outs.

4.2.13 Statistical Analysis

Data was analyzed using the Statistical Analysis System (SAS) for Windows (SAS Institute Version 8.2, Cary NC, USA). Parameters measured were expressed as mean± standard deviation. Mean and standard deviation was calculated for age, weight, height, BMI, nutrient intake, vision parameters, individual fatty acids (AA and DHA in plasma and RBC phospholipids) for all subjects. Two-way analysis of variance (ANOVA) was used to test for effect of treatment, time (0 and 7 months), and any interactions with age, weight, height, BMI, nutrient intakes, vision parameters, and individual fatty acids (AA and DHA in plasma and RBC phospholipids). Within each treatment group, t tests were used to compare the effect over time (0 and 7 months) in individual fatty acids (AA and DHA in plasma and RBC phospholipids).

A three-way ANOVA was used to test for the effect of treatment, on DHA intakes assessed by food records and food frequency questionnaire, time (0 and 7 months), and any interactions between treatment, DHA intakes (assessed by food records and food

frequency questionnaire), and time. Repeated measures ANOVA was used to assess the effect of time (0, 3, 7 months), treatment, and interactions on nutrient intakes. Nonparametric Wilcoxon Rank-Sum test (Mann Whitney t test) was used to test the effect of treatment on the TVPS-R test parameters (visual perceptual skills subsets, sum of scaled scores, and median visual perceptual age). Wilcoxon signed rank test was used to assess the effect of time (0 and 7 months) within each treatment group for TVPS-R test parameters (visual perceptual skills subsets, sum of scaled scores and median visual perceptual age). P values <0.05 were considered significant. DHA intake (mg/day) at 7 months and median visual perceptual age (months) was compared as a multivariate analysis using the linear discriminate function procedure in S-plus for Windows Professional Edition (Version 6.2.1, Seattle, Washington, USA) and was plotted graphically. The equation for the discriminate function was obtained by solving the pooled covariance matrix of the two variables (DHA intake and median visual perceptual age) and the mean difference vectors between the two groups using linear programming (SAS Institute Version 8.2, Cary NC, USA).

Fatty acid content of the supplement was rounded to three significant figures, means and ranges were rounded to three significant figures. Standard deviations were rounded to either three significant figures (nutrient, vitamin and mineral intakes) or to one decimal place.

4.3 Results

4.3.1 Subjects

In a previous study (Chapter 3), 63 out of the 78 subjects met inclusion criteria to participate in the present study. A total of 37 subjects were recruited, of which 26 (F=12, M=14; LCP=12, control=14) completed the entire study protocol (70% completion rate). Eleven children dropped out (LCP group=8, control=3). Eight children did not comply with the protocol, one child did not like the taste of the supplement, and two children dropped out due to both non compliance and dislike of the supplement. Mean age of all children was determined from the date the first blood/ophthalmic test was preformed as 6.5±0.8 years (range 4.5-7.6 years). Age of the subjects in the supplemented group (n=12) was 6.3±0.8 years and age in control group (n=14) was 6.6±0.7 years. There was no significant difference in age between the two treatment groups.

4.3.2 Formula Intake

Treatment groups had similar mean total intake of sachets (Control group: 555±73.3 and LCP group: 499±69.1) and mean total days on study period (Control group: 228±28.5 days, LCP group 248±39.7 days), which allows for comparability between both treatment groups. Total amount of AA and DHA consumed from the supplement by the LCP group during the course of the study from the supplement was 5.19±0.7 g of AA and 3.49±0.5 g of DHA.

4.3.3 Growth

Treatment did not affect the growth patterns (weight, height, BMI) of children (Table 4.1). There was a significant increase in weight, height, and BMI in all children over time, as can be expected as children are growing at this age. Gender was not a significant factor in the analysis and interaction between treatment groups (LCP and Control group) and collection time (0 and 7 months) was not significant.

Table 4.1 Weight, Height, and Body Mass Index of Subjects in Control and LCP Groups at 0 and 7 months

	Control		LCP		Significant effects[2] (P values, Pr>F)	
	0 months	7 months	0 months	7 months	Main effects	
Test groups	(n=14)	(n=14)	(n=12)	(n=12)	Treatment	Collection time
Weight (kg)	24.5 (2.6)[1]	28.4 (2.9)	23.5 (4.4)	26.6 (5.8)	NS	<0.0001
Height (cm)	120 (5.2)	125 (4.3)	118 (6.9)	123 (6.3)	NS	<0.0001
Body mass index (kg/m^2)	16.9 (1.8)	18.2 (1.9)	16.7 (2.5)	17.6 (3.2)	NS	<0.0001

[1]Values represent mean ± (standard deviation)
[2]When tested as a main effect, gender was not a significant factor in the analysis; there were no significant interactions.

4.3.4 Diet Assessment

Food Records

Daily nutrient intake was estimated from 4-day food records from control and LCP group at 0, 3, and 7 months (Table 4.2). Macronutrient (protein, carbohydrate, and fat) intake met the acceptable macronutrient distribution ranges (AMDR) suggested by the Institute of Medicine (2002). Fat intakes were between 25-35% of energy, carbohydrate was between 45-65% of energy, and protein intakes were 10-30% of energy, for all children specified by the AMDR for those 4-18 years of age (Institute of

Medicine 2002). A total of 10 of 14 subjects in the control group reduced their food intake over time when consuming the control supplement to compensate for the additional calories provided by the control supplement. Half of the subjects (6 of 12) in the LCP group reduced their food intake while consuming the LCP supplement to compensate for the additional calories provided by the LCP supplement. Of the remaining 6 subjects in the LCP group, 5 subjects increased their food intake while consuming the LCP supplement, and 1 subject maintained his level of food intake. Using repeated measures ANOVA to assess the effect of treatment and time (0, 3 and 7 months), consumption of either formula (control and LCP packets) resulted in an increase in total energy intake over time in both treatment groups. However, when caloric intake was assessed on a per body weight basis at 0 and 7 months, there were no significant differences between treatment groups or over time. Fiber intake in this age group was lower than the recommended adequate intake (AI) for 4-8 year olds (25 g/day) (Institute of Medicine 2002).

Average intake of vitamin and minerals (Table 4.2) met the recommended estimated average requirement (EAR) and AI, for this age group (Institute of Medicine 1997; 1998; 2000; 2001; 2004). Consuming the supplement significantly increased intakes of calories, protein, fat, riboflavin, vitamin D, vitamin E, pantothenic acid, calcium, iron, magnesium, phosphorus, selenium, and zinc over time. When nutrients were assessed per body weight basis over time, vitamin D and calcium was significantly increased over time.

Consumption of the supplement in both control and LCP group increased intake of palmitic acid, oleic acid, LA, ALA, AA, and overall omega 3 and omega 6 intake over

time (Table 4.3). In 24 of the total 26 subjects at 0 months the LA intake was lower than the AI for LA (10 g/day) for children 4-8 years of age (Institute of Medicine 2002). Post supplementation at 7 months improved LA status in 10 subjects, therefore 13 of the total 26 subjects had lower LA intake compared to the AI for LA (Institute of Medicine 2002). In 19 of the total 26 subjects at 0 months ALA intake was lower than the AI for ALA (0.9 g/day) for children 4-8 of age (Institute of Medicine 2002). Post supplementation at 7 months improved ALA status in 4 subjects, therefore 14 of the total 26 subjects that had lower ALA intakes compared to the AI for ALA (Institute of Medicine 2002).

Table 4.2 Daily Nutrient, Vitamin and Mineral Intake from Food Consumption Assessed Using 4-day Food Record at 0 and 7 Months of Subjects in Control and LCP groups[1]

	All children 0 months	Control 7 months	LCP 7 months	Significant effects (P values, Pr>F)[3, 4] Main effects	
Nutrients per day	(n=26)	(n=14)	(n=12)	Treatment	Collection time
Calories (kcal)	1790(415)[2]	2010(260)	1970(393)	NS	>0.01
Protein (g)	58.0(13.8)	66.8(14.0)	68.2(26.8)	NS	>0.01
% of total kcal	13.1(1.9)	13.2(1.5)	13.6(3.2)	NS	NS
Protein (g)/ 1000 kcal	32.7(4.8)	33.1(3.6)	34.1(8.1)	NS	NS
Carbohydrate (g)	252(69.8)	268(35.1)	256(55.8)	NS	NS
% of total kcal	56.4(6.3)	53.6(6.4)	52.7(9.8)	NS	<0.05
Carbohydrate (g)/1000 kcal	141(15.7)	134(15.9)	132(24.4)	NS	<0.0001
Fat (g)	64.6(16.3)	76.8(16.8)	77.3(23.8)	NS	<0.01
% of total kcal	32.5(4.6)	34.2(4.9)	35.0(6.4)	NS	NS
Fat (g)/ 1000 kcal	32.5(4.6)	38(5.4)	38.9(7.2)	NS	NS
Fiber (g)	12.2(3.4)	11.3(2.5)	10.9(4.2)	NS	NS
Cholesterol (mg)	184(91.5)	163(68.1)	236(197)	NS	NS
Vitamin A (IU)	6500(4610)	7160(4380)	7440(5570)	NS	NS
Vitamin A (RE)	957(465)	863(404)	961(537)	NS	NS
Thiamin B1 (mg)	1.58(0.7)	1.7(0.3)	1.64(0.4)	NS	NS
Riboflavin B2 (mg)	1.70(0.8)	2.01(0.2)	1.84(0.5)	NS	<0.05
Niacin B3 (mg)	20.1(7.1)	17.5(5.1)	17.0(5.9)	NS	NS
Niacin B3-NE (mg)	24.6(6.8)	22.4(6.2)	22.1(8.7)	NS	NS
Vitamin B6 (mg)	1.71(1.0)	1.56(0.3)	1.30(0.4)	<0.05	NS
Vitamin B12 (mcg)	3.23(2.4)	3.21(1.8)	3.33(1.4)	NS	NS
Vitamin C (mg)	120(93.1)	116(32.6)	87.3(34.1)	NS	NS
Vitamin D (IU)	270(141)	379(78.1)	390(123)	NS	<0.001
Vitamin D (mcg)	5.33(1.9)	9.46(2.0)	9.54(3.2)	NS	<0.0001
Vitamin E (IU)	9.65(8.9)	11.1(1.5)	10.8(2.7)	NS	NS
Vitamin E (mg)	7.03(3.8)	10.7(3.3)	8.80(2.3)	NS	<0.001
Folate (mcg)	281(91.1)	242(46.5)	240(75.8)	NS	<0.05
Pantothenic acid (mg)	4.35(3.7)	3.77(0.5)	3.63(1.2)	NS	NS
Calcium (mg)	873(263)	1310(311)	1270(287)	NS	<0.0001
Copper (mg)	0.968(0.4)	0.886(0.2)	0.873(0.2)	NS	NS
Iron (mg)	13.7(5.0)	17(3.2)	15.0(3.5)	<0.05	<0.001
Magnesium (mg)	178(57.4)	202(28.2)	177(33.2)	<0.01	<0.05
Manganese (mcg)	2.21(0.8)	1.77(0.5)	1.88(0.7)	NS	<0.05
Phosphorus (mg)	869(279)	1110(149)	1050(309)	NS	<0.01
Selenium (mcg)	57.6(17.2)	62.8(14.7)	63.5(19.6)	NS	<0.05

Sodium (mg)	2860 (703)	2790 (603)	2870 (779)	NS	NS
Zinc (mg)	8.18 (2.9)	9.48 (2.4)	8.47 (3.1)	<0.05	NS

[1]Food records were taken at 0, 3, and 7 months for all subjects. Supplement packets were consumed at 3 and 7 months and were recorded in the 4-day food records as part of the diet. Food records at 3 months were not significantly different from food records at 0 and 7 months.
[2]Values represent mean ± (standard deviation)
[3]When tested as a main effect, gender as not a significant factor in the analysis, except in fiber, manganese and sodium. There were no significant interactions, except for fiber and manganese where both gender and supplement was significant at $P < 0.05$.
[4]Nutrients were also assessed on a per body weight basis at 0 and 7 months using a two-way ANOVA. There were no significant differences between treatment groups when nutrients were assessed on a per body weight basis. Nutrients that were significantly different over time at $P<0.05$ include vitamin B6, %fat of total kcal, and fat/1000 kcal. Nutrients that were significantly different over time at $P<0.01$ include % protein of total kcal, protein/1000 kcal, fiber, niacin B3, niacin B3-NE, folate, calcium, and manganese. Nutrients that were significantly different over time at $P<0.0001$ include % carbohydrate of total kcal, carbohydrate/1000 kcal vitamin D (mcg) <0.0001. There were no significant interactions between treatment groups (control and LCP group) and time (0 and 7 months).

Table 4.3 Daily Fatty Acid Intake from Food Consumption Assessed using 4-day Food Record at 0 and 7 Months of Subjects in Control and LCP groups[1]

Fatty acid per day	All children 0 months (n=26)	Control 7 months (n=14)	LCP (excluding AA and DHA supplement) 7 months (n=12)	Significant effects (P values, Pr>F)[3,4] Main effects Treatment	Collection time
Palmitic acid (16:0) (g)	9.6 (2.9)	13.3 (3.9)	11.8 (4.9)	NS	<0.01
Stearic acid (18:0) (g)	4.80 (1.4)	5.34 (1.9)	5.23 (2.3)	NS	NS
Oleic acid (18:1) (g)	17.3 (4.1)	24.5 (6.0)	22.0 (9.94)	NS	<0.01
Linoleic acid (18:2) (g)	6.5 (2.9)	9.92 (2.1)	8.12 (1.9)	NS	<0.0001
Linolenic acid (18:3) (g)	0.697 (0.3)	0.965 (0.2)	0.831 (0.3)	NS	<0.0001
Arachidonic acid (20:4) (mg)	49.7 (39.2)	67.4 (46.2)	78.8 (78.9)	NS	<0.05
Eicosapentaenoic acid (20:5) (mg)	23.1 (41.0)	4.81 (5.9)	7.84 (10.2)	NS	<0.05
Docosahexaenoic acid (22:6) (mg)	36.2 (42.7)	16.4 (13.8)	26.6 (23.9)	NS	NS
Omega 3 (g)	0.746 (0.3)	0.985 (0.2)	0.864 (0.3)	NS	<0.001
Omega 6 (g)	6.38 (2.9)	9.87 (2.1)	7.85 (1.8)	NS	<0.0001

Fatty acid per day	All children	Control	LCP (including AA and DHA supplement)	Significant effects (P values, Pr>F)[3,5]	
Arachidonic acid (20:4) (mg)	49.7 (39.2)	67.4 (46.2)	102 (34.3)	NS	<0.01
Docosahexaenoic acid (22:6) (mg)	36.2 (42.7)	16.4 (13.8)*	42.2 (25.0)*	NS	NS
Omega 3 (g)	0.746 (0.3)	0.985 (0.2)	0.880 (0.3)	NS	<0.001
Omega 6 (g)	6.38 (2.9)	9.87 (2.1)	7.87 (1.8)	NS	<0.001

[1]Food records were taken at 0, 3, 7 months for all subjects. Supplement packets were consumed at 3 and 7 months and were recorded in the 4-day food records as part of the diet. Food records at 3 months were not significantly different from food records at 0 and 7 months.
[2]Values represent mean ± (standard deviation)
[3]When tested as a main effect, gender as not a significant factor in the analysis. There were no significant interactions.
[4]Nutrients were also assessed on a per body weight basis at 0 and 7 months using a two-way ANOVA. There were no significant differences between treatment groups when nutrients were assessed on a per body weight basis. Nutrients that were significantly different over time at P<0.05 include LA and omega 6 (LCP excluding AA and DHA supplement). There were no significant interactions between treatment groups (control and LCP group) and time (0 and 7 months).

[5]Nutrients were also assessed on a per body weight basis at 0 and 7 months using a two-way ANOVA. There were no significant differences between treatment groups when nutrients were assessed on a per body weight basis. AA ($P<0.01$) and omega 6 (0.05) was significantly different over time (LCP including AA and DHA supplement). There were no significant interactions between treatment groups (control and LCP group) and time (0 and 7 months).

* Treatment groups were assessed using T-Test at 7 months. The LCP group (including AA and DHA supplement) had a higher intake of DHA at 7 months ($P<0.01$) compared to the control group at 7 months.

AA intake from the diet was not different between treatment groups (control and LCP group), however AA intake from the diet increased over time (0, 3, 7 months) (Table 4.3). DHA intake from the diet was not different between treatment groups (control and LCP group) or over time (0, 3, 7 months). Median intake of AA for the control group was 53.9 mg/day, 48.2 mg/day, 71.9 mg/day and DHA was 14.4 mg/day, 14.5 mg/day and 14.6 mg/day at 0, 3, and 7 month respectively. Median intake of AA for the LCP group (not including AA and DHA from the supplement) was 40.9 mg/day, 59.2 mg/day, 55.3 mg/day, and for DHA was 23.7 mg/day, 14.7 mg/day and 20.3 mg/day respectively. Median intake of AA for the LCP group (including the AA and DHA supplement) was 82.8 mg/day, 78.7 mg/day and for DHA was 32.2 mg/day, 36.9 mg/day at 3 and 7 months respectively.

AA intake from food consumption alone (excluding formula) ranged from 3.2-356 mg/day in the control group and 7.24-354 mg/day in the LCP when intakes were calculated per day using the 4-day food record over the 7 month period. DHA intake from food consumption alone (excluding formula) ranged from 0-377 mg/day in control subjects and 0-403 mg/day in LCP subjects when intakes were calculated per day using the 4-day food record over the 7 month period. The range of AA and DHA intake (per day basis using 4-day food record) is evidence of intra and inter individual variation in DHA and AA intake in both the control and LCP group (Figure 4.1 and Figure 4.2).

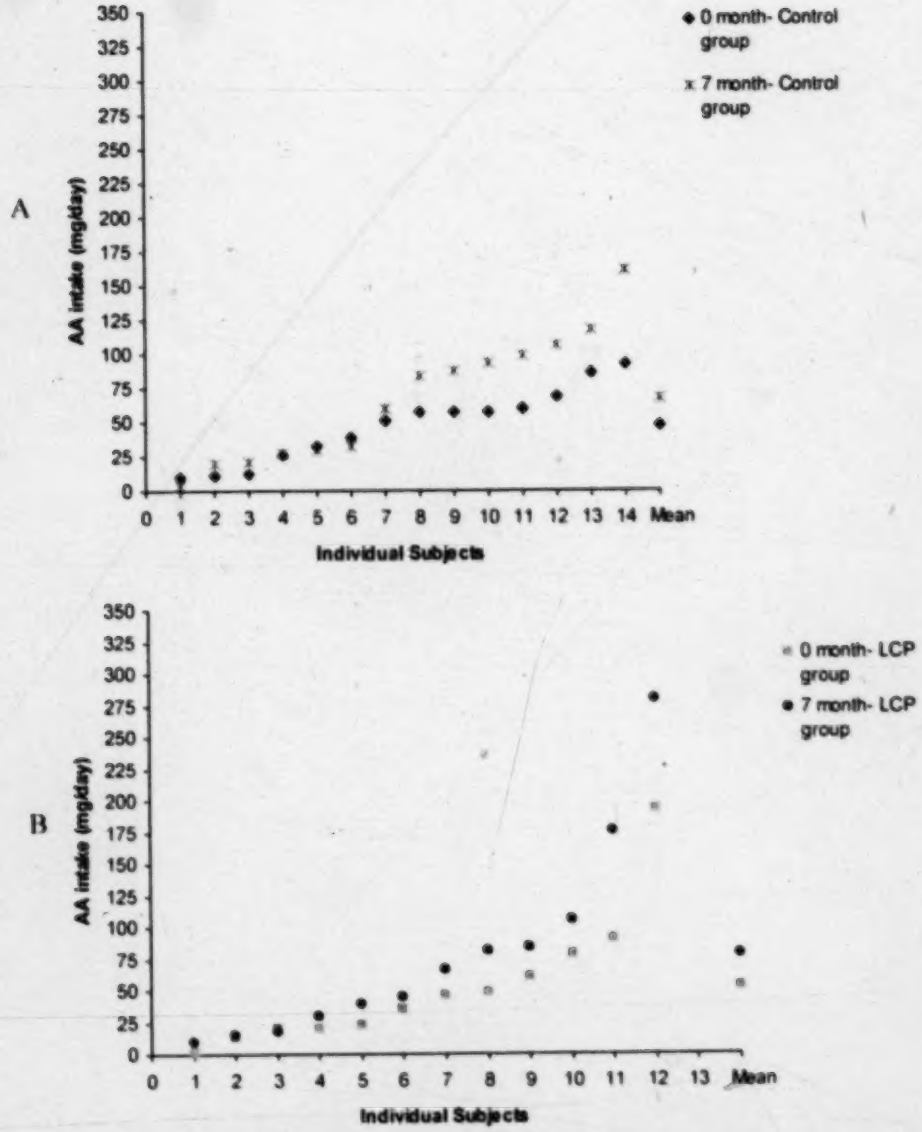
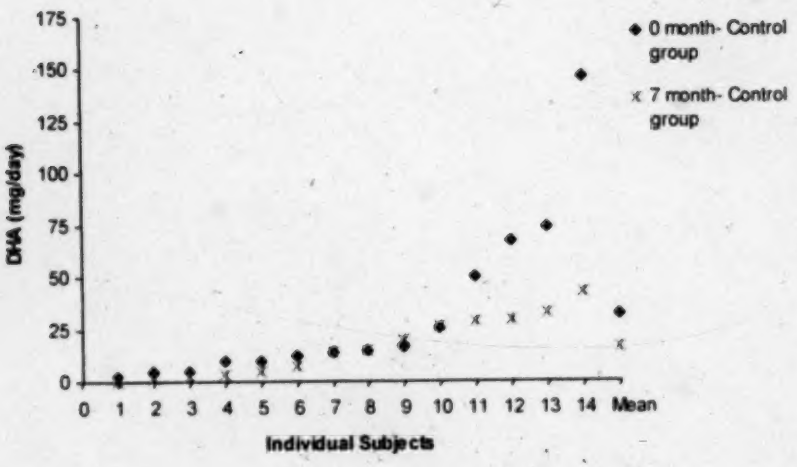


Figure 4.1 Mean Individual Intakes of AA (mg/day) from Food Consumption Assessed by 4-day Food Records at 0 and 7 months in Control (A) and LCP subjects (B)

A



B

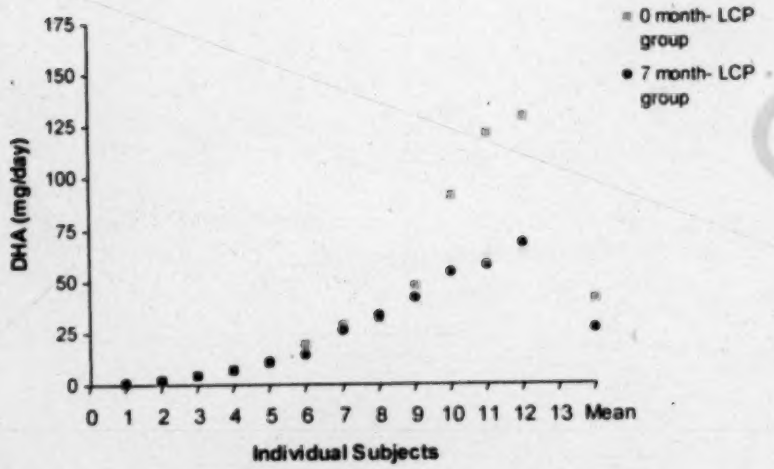


Figure 4.2 Mean Individual Intakes of DHA (mg/day) from Food Consumption Assessed by 4-day Food Records at 0 and 7 months in Control (A) and LCP subjects (B)

DHA Intakes Assessed by Food Frequency Questionnaire

DHA intake (mg/day) assessed using a food frequency questionnaire includes DHA from food sources only and does not take into account DHA intake from the supplement for the LCP group. DHA intake (mg/day) when assessed with food frequency questionnaire alone was not significantly different between treatment groups, however DHA intake significantly decreased over time (Table 4.4). A wide range of DHA intake (mg/day) was observed using the food frequency questionnaire at 0 and 7 months in the both treatment groups.

Table 4.4 Comparison of Docosahexaenoic Acid Intake Determined from 4-day Food Record and Food Frequency Questionnaire at 0 and 7 Months in Control and LCP subjects[1]

	Food Frequency Questionnaire				Food record				Significant effects (P values, Pr>F) Main effects[3] Control vs. LCP (excluding AA and DHA supplement)		
	Control		LCP		Control		LCP (excluding AA and DHA supplement)				
	0 months	7 months	0 months	7 months	0 months	7 months	0 months	7 months			
Variables	(n=14)	(n=14)	(n=12)	(n=12)	(n=14)	(n=14)	(n=12)	(n=12)	Treatment	Food assessment methods	Collection time
DHA intake (mg/day)	26.7 (14.5)[2]	23.8 (13.3)	47.4 (36.8)	31.4 (18.1)	32.4 (40.2)	16.4 (13.8)	40.7 (46.9)	26.6 (23.9)	NS	NS	<0.05
Range of DHA intake (mg/day)	12.4-58.7	8.77-58.0	7.29-117	2.99-68.2	2.06-146	0.00-43.1	0.00-129	0.690-68.2	-	-	-

[1]DHA intake for food frequency questionnaire and 4-day food records include intake records taken at both 0 and 7 months.
[2]Values represent mean ± (standard deviation)
[3]When tested as a main effect, gender was not a significant factor in the analysis; there were no significant interactions

Comparison of DHA Intakes Assessed by Food Frequency Questionnaire and Food Record

The DHA intakes (mg/day) assessed from both food frequency questionnaire and food record, at 0 and 7 months in control and LCP group are reported (Table 4.4). DHA intakes assessed by the food record at month 7 excludes the AA and DHA from the supplement, allowing for determination of DHA intake in the LCP group from food sources other than that provided by the supplement. This allows for comparison of DHA intake in food sources only (other than that provided by the supplement), between food frequency questionnaires and food records at 0 and 7 months (Table 4.4).

There is a wide range of DHA intakes (mg/day) reported from both food assessment methods (food record and food frequency questionnaire), and there is agreement between these two food assessment methods (P=NS). It appears that providing the supplement is associated with a reduction in the intake of DHA containing foods from both the control and LCP group over time ($P<0.05$).

4.3.5 Visual Function Assessments

The HOVT test in both the left eye (control: 0 months 42.6±1.6, 7 months 43.1±2.2; LCP: 0 months 42.7±2.2, 7 months 42.4±2.5) and the right eye (control: 0 months 42.6±1.6, 7 months 43.0±2.1; LCP: 0 months 42.2±3.3, 7 months 42.3±2.2) was not different between treatment groups or over the 7 months. The colour test in both the left eye (control: 0 and 7 months 7.64±1.1; LCP: 0 and 7 months 8.00±0.0) and the right eye (control: 0 months 7.64±1.1, 7 months 7.50±1.3; LCP: 0 months 8.00±0.0; 7 months 7.92±0.3) was not different between treatment groups or over the 7 months. Cyclo

refraction in both the left eye (control: 0 months 0.723±0.8, 7 months 0.464±0.6; LCP: 0 months 1.41±1.9, 7 months 1.26±1.7) and the right eye was not different between treatment groups (control: 0 months 0.741±0.8, 7 months 0.420±0.6; LCP: 0 months 1.22±1.5, 7 months 1.03±1.2). There were no differences in cyclo refraction over the 7 months in the left eye; however, there was a decrease in cyclo refraction in the right eye over the 7 months over time ($P<0.05$). Stereo acuity and the prism test was not different between the two treatment groups or over the 7 months.

4.3.6 Neurological Assessment

Using nonparametric Wilicoxon Rank-Sum, TVPS-R parameters (7 subsets include: visual discrimination, visual memory, visual spatial-relationships, visual form-constancy, visual sequential memory, visual figure-ground, visual closure), scaled scores, and median visual perceptual age was not different between treatment groups (data not shown). When TVPS-R subsets were assessed within the control group, visual sequential memory (1 of 7 subsets) increased over the 7 months. When TVPS-R subsets were assessed within the LCP group, visual discrimination, visual spatial relationships, and visual figure group (3 of 7 subsets) increased over the 7 months. Overall TVPS-R scaled scores and median visual perceptual age within the LCP group also increased over time (Table 4.5).

When DHA intake (mg/day) at 7 month was plotted against median visual perceptual age (months) using linear discriminate analysis (a multivariate procedure), an equation was derived to separate subjects in the two treatment groups. The equation can be used to predict which group an individual may belong to based on DHA intake and

median visual perceptual age. The line, derived from the linear discriminate analysis, separates the two groups, that is the Control and LCP group. Only two subjects in the control group are found above the derived line, and five subjects from the LCP group are found below the derived line (Figure 4.3), suggesting that the treatment resulted in two groupings of responses.

Table 4.5 Mean and Standard Deviation of Variables from the TVPS-R at 0 and 7 Months Comparisons of Subjects in Control and LCP groups

Variables	Control group (n=14)		*P value	LCP group (n=12)		*P value
	0 months	7 months	Collection time	0 months	7 months	Collection time
1. Visual discrimination	8.86 (3.6)[1]	10.1 (4.5)	NS	10.8 (3.2)	12.3 (3.1)	<0.05
2. Visual memory	8.86 (3.6)	10.4 (2.2)	NS	10.5 (2.4)	11.8 (2.2)	NS
3. Visual spatial-relationships	10.1 (5.4)	11.4 (5.7)	NS	12.2 (3.2)	13.9 (2.3)	<0.05
4. Visual form-constancy	8.29 (3.2)	9.57 (4.1)	NS	8.50 (2.6)	9.75 (3.3)	NS
5. Visual sequential-memory	5.29 (3.3)	8.86 (4.8)	<0.05	7.17 (5.2)	8.33 (6.0)	NS
6. Visual figure-ground	7.93 (4.1)	9.64 (4.1)	NS	9.17 (4.4)	13.0 (2.3)	<0.05
7. Visual closure	5.29 (3.8)	6.86 (4.1)	NS	7.25 (2.9)	9.08 (4.1)	NS
Sum of scaled scores	59.6 (16.6)	67.4 (22.1)	NS	76.3 (17.3)	82.1 (18.6)	<0.05
Median visual perceptual age (months)	75.2 (23.0)	91.9 (37.2)	NS	85.6 (24.0)	112 (37.0)	<0.01
Median visual perceptual age (years)	6.27 (1.91)	7.65 (3.10)	NS	7.13 (2.00)	9.33 (3.08)	<0.01

[1]Values represent mean ± (standard deviation)

Caption: Nonparametric statistics, specifically the Wilicoxon signed rank test was used to assess the effect of time (0 and 7 months) within each treatment group (Control and LCP group) for the TVPS-R test parameters (visual perceptual skills subsets, sum of scaled scores, and median visual perceptual age).

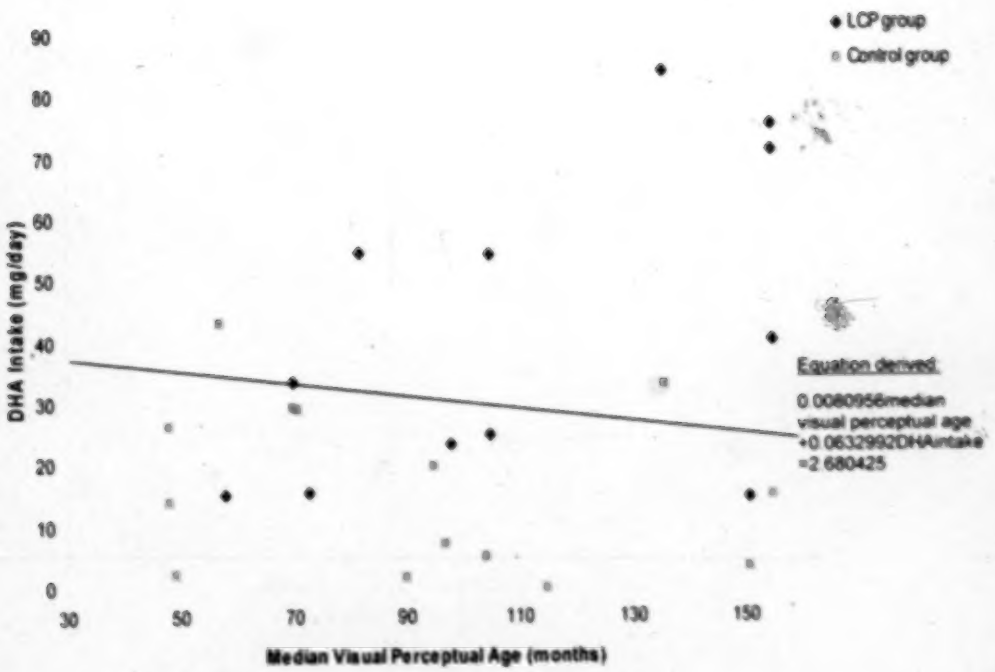


Figure 4.3 Total DHA Intake (mg/day) plotted versus Median Visual Perceptual Age (months) at 7 months

Caption: Total DHA intake (from food sources and supplement) was plotted against median visual perceptual age at 7 months. Using linear discriminate function, an equation was derived which can be used to predict which group an individual may belong to based on DHA intake and median visual perceptual age. The line separates the two treatment groups and shows that there is an effect of treatment, as the control and LCP group are distributed differently.

4.3.7 Individual Fatty Acids (AA and DHA in plasma phospholipids and RBC phospholipids)

AA (%wt/wt) in the red blood cell (RBC) phospholipid (control 0 months 7.6±2.6, 7 months 6.8±2.6; LCP 0 months 8.1±2.4, 7 months 8.3±2.8) and DHA (%wt/wt) in the RBC phospholipid (control 0 months 2.9±2.5, 7 months 1.8±0.9; LCP 0 months 2.8±2.0, 7 months 2.1±1.2) was not different between the two treatment groups when compared by ANOVA. The mean AA level (ug/mL) in the plasma phospholipid fraction (control 0 months 61.6±14.4, 7 months 57.1±17.2; LCP 0 months 61.8±12.9, 7 months 68.6±24.5) and mean DHA level (ug/mL) in the plasma phosholipid fraction (control 0 months 16.7±5.1, 7 months 13.8±4.4; LCP 0 months 16.4±3.1, 7 months 16.2±5.1) was not different between the two treatment groups or over time when compared by ANOVA. Using a T-test both AA (%wt/wt) in the RBC phospholipid and DHA (%wt/wt) in the RBC phospholipid was not significantly different within treatment groups (control and LCP) over time. AA level (ug/mL) in the plasma phospholipid fraction and DHA level (ug/mL) in the plasma phospholipid fraction was not significantly different within treatment groups (control and LCP) when assessed over time using a T-test. When AA and DHA content in the RBC phospholipid and the plasma phospholipids was plotted for all subjects, there was a general trend toward a higher level of AA and DHA in the LCP group compared to the control group at 7 months (Figure 4.4 and Figure 4.5).

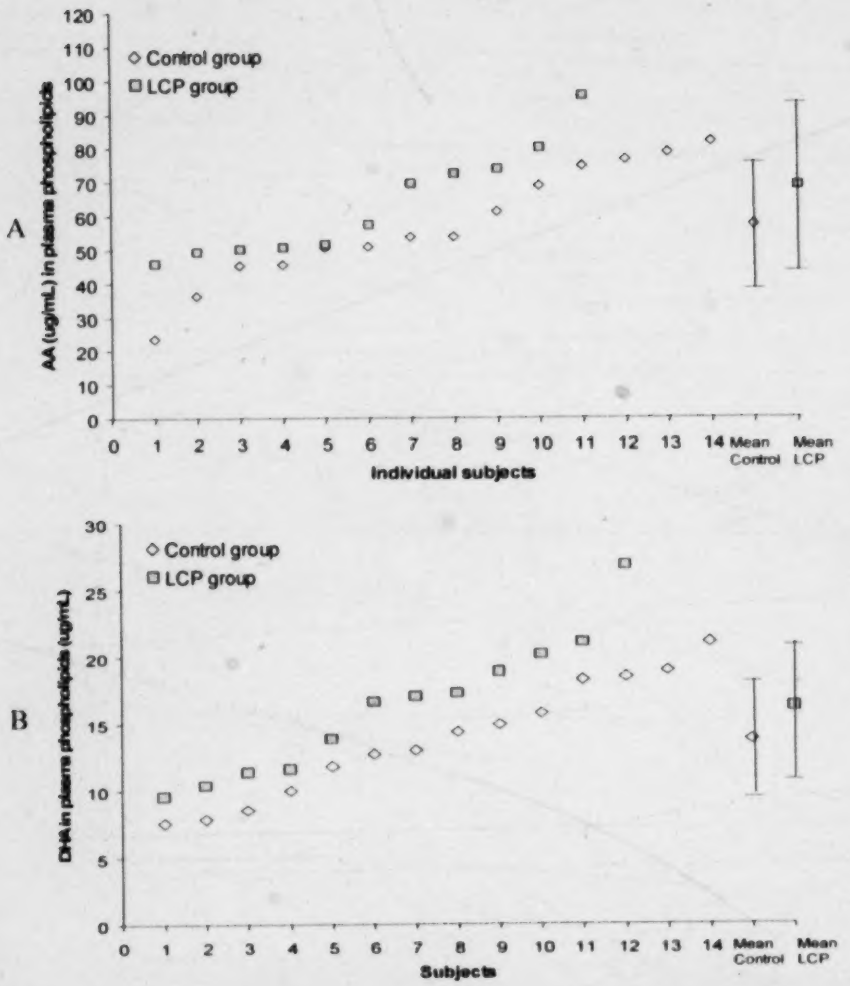


Figure 4.4 AA (A) and DHA (B) level (ug/mL) in Plasma Phospholipids in Control and LCP Subjects at 7 months

Caption: The bars on the mean denote the standard deviation for the control group and LCP group.

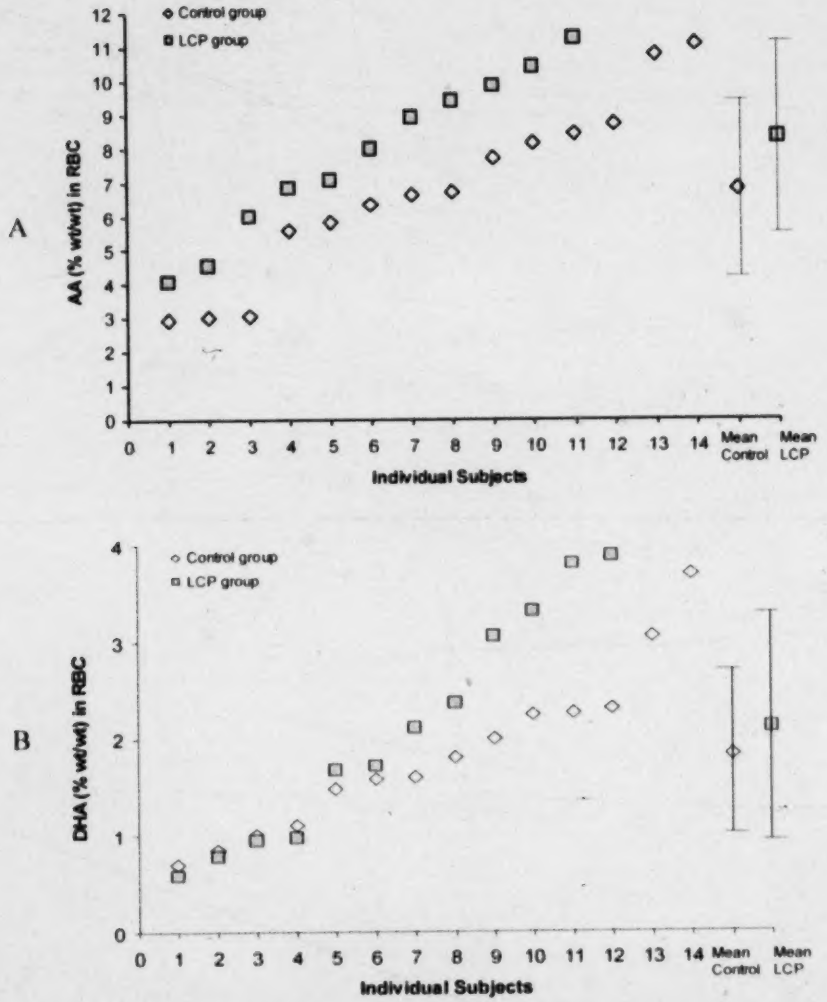


Figure 4.5 AA (A) and DHA (B) level (%wt/wt) in Phospholipids of Red Blood Cells in Control and LCP Subjects at 7 Months.

Caption: The bars on the mean denote the standard deviation for the control group and LCP group.

4.3.7 Health Diary

The incidence of colds, flu, headaches, sore throat, and cough, was not different between children in either group. Consuming the supplement did not result in any serious adverse events and was tolerated during the study period.

4.4 Discussion

To our knowledge there are no studies that have supplemented healthy children with AA and DHA to assess the effect on essential fatty acid status and visual perception. In the present study, 5-7 year old children (range 4.5-7.6 years), previously determined to consume low intakes of DHA were provided with a nutritionally complete dietary supplement containing AA and DHA for a period of 7 months. Supplementation with AA and DHA, showed a trend towards increased essential fatty acids (AA and DHA content of phospholipids RBC and plasma phospholipids) and improvement in visual perception.

4.4.1 Diet Assessment

Macronutrient, Vitamin and Mineral Intakes

Both LCP and control groups achieved nutrient intakes for macronutrients, vitamins and minerals recommended for the AMDR, EAR and AI by the Institute of Medicine (1997; 1998; 2000; 2001; 2002; 2004) (Table 4.2). Vitamin A, vitamin E, and iron intakes met the EAR levels which indicates that the children studied were not at risk of having inadequacies of these nutrients, and any effect on vision or visual perception may be the result of taking the formula.

Providing a nutritionally complete dietary supplement to both the control and LCP group did not increase overall energy intake of the children during the 7 month study (Table 4.2) when assessed on a per body weight basis. Majority of the children reduced their food intake while consuming the dietary supplement to compensate for the additional calories provided by the dietary supplement, therefore taking the dietary supplement will not likely cause unhealthy weight gain in children since the supplement did not increase overall energy intakes.

Dietary supplementation with the nutritionally complete formula, lead to increase in LA and ALA status over the 7 months in all children. Although there was an improvement in the intake of LA and ALA post supplementation, 50% and 53% of all children studied have intake lower than the AI for LA and ALA respectively. Recent stable isotope studies have suggested that preterm and term infants (Carnielli et al. 1996; Salem et al. 1996; Sauerwald et al. 1996; Szitanyi et al. 1999), and human adults (Burdge et al. 2003; Burdge et al. 2002; Salem et al. 1999) have low ability to convert LA to AA and ALA to DHA. The extent to which de novo synthesis of these LCP are sufficient to support the needs of the growing child has not been investigated. Stable isotope studies have shown that in human adults there is limited conversion of ALA to DHA (Emken et al. 1994; Hussein et al. 2005). When preterm infants were fed formulas without AA and DHA, plasma levels of AA and DHA in the phospholipid fractions declined (Clandinin et al. 1997).

Food Assessment Method used to Assess AA and DHA Intake

The food assessment method used to assess AA and DHA intake contributed to the wide variability of AA and DHA intake that was observed. In the present study, diet was assessed using a 4-day unweighed food record. Use of a 7-day weighted food record may be considered a more precise method for estimating usual food and/or nutrient intakes of individuals, as long as respondents do not change their usual eating pattern (Gibson 1990). This method was considered to be too much of a burden to parents participating in the present study and could have resulted in problems with compliance and completion rates. Subjects in the present study were recruited from a previous study, where nutrient intakes of AA, DHA, vitamin A, vitamin E, and iron were assessed using a 3-day unweighed food record (Lien and Clandinin unpublished). Parents were familiar with using a 3-day unweighed food record, therefore the inclusion of an additional day (4-day food record) for the present study would provide further information on usual food and nutrient intakes of their child.

In the present study, a food frequency questionnaire was also used to assess DHA intake in the same children. Both the food record and the food frequency questionnaire in the present study showed agreement when assessing DHA intakes, and showed that providing a supplement to the children, resulted in reduced intake of DHA containing foods in all children. Food frequency questionnaires provide an inferior quantitative estimate of intake as this method does not usually define information on a specific food item consumed, exact portion sizes consumed, food preparation methods, brand name and packaging information (Allison et al. 1999; Briefel et al. 1992; Sempos 1992). In the present study and in a study by Innis et al. (2004) who assessed AA and DHA intake

using a food frequency questionnaire, these limitations were considered and data was collected on specific foods (e.g. frequency food was eaten, portion sizes, method of preparation, and brand name).

AA and DHA Intakes

Dietary assessment of DHA intake using both 4-day food records and food frequency questionnaires confirmed that current dietary intake of DHA was low and ranged widely (Table 4.3 and Table 4.4). This was expected as foods containing rich sources of DHA such as fatty fish are not consumed on a daily basis by the children studied, therefore intra and inter subject variation in AA and DHA intakes exists (Figure 4.1 and 4.2). This is the first study to show that consumption of 2-3 sachets/day resulted in decreased intakes of DHA containing foods over time in both treatment groups when assessed by both food records and food frequency questionnaires. This was an unexpected outcome that complicates interpretation of findings, as it appears that dietary habits may change as a result of consuming the supplement. Supplementation also resulted in increased AA intake over time in both treatment groups, but was not significantly different between treatment groups when assessed using ANOVA.

Few studies report AA and DHA intake in children. Of these studies, the data reported varies (Allison et al. 1999; Innis et al. 2004; Jonnalagadda et al. 1995; Meyer et al. 2003). In Australian children, 4-7 years of age (n=799), AA intakes were lower (22 mg/day) while DHA intakes were higher (47 mg/day) than the AA and DHA intakes reported in the present study. In US children, 6-11 years of age (Jonnalagadda et al. 1995) and children 3 years of age and older (Allison et al. 1999) estimated higher 18:4+AA (100 ±0.01 mg/day) and 20:5+DHA (100 ±0.01 mg/day) intake than the AA

and DHA intakes assessed in the present study. Similarly, Innis et al. (2004) reported higher levels of AA (226±17 mg/day) and DHA (96±14 mg/day) intake in 3-5 year old Canadian children.

The geographic location where the food assessments were completed is one of the reasons why wide variability of AA and DHA intake was observed. Many of the nutrient assessments were national surveys (Allison et al. 1999; Jonnalagadda et al. 1995; Meyer et al. 2003) which provide the mean intakes of AA and DHA for all subjects in the study. Although it is important to estimate intakes of AA and DHA intake consumed by a population, it may not indicate areas or subject groups that have lower intake of these fatty acids. DHA intakes assessed in preschool children living in the Vancouver Costal Health Authority Region, British Columbia, Canada are expected to have higher intakes of DHA than those reported in the present study, as seafood is readily available in the area (Innis et al. 2004).

4.4.2 Visual Function Assessments

Complete ophthalmic exams were required to assess visual status in all the children prior to supplementation. The visual assessments confirmed that supplementation did not have an adverse effect on vision and was an important safety parameter. Previous studies done on infants and toddlers in relation to DHA intakes alone (Williams et al. 2001), or DHA and AA intakes (Agostoni et al. 1997; Agostoni et al. 1995) have assessed either vision or neurological aspects, but have not combined both these measures. When assessing neurological development it is important to determine if

the score derived is the result of poor LCP status or poor vision if the subject cannot clearly see the object they are to identify, as both of these factors are intertwined.

4.4.3 Neurological Assessment

In the present study, the TVPS-R test showed that when LCP subjects were given a daily dose of AA and DHA in addition to their dietary intake, there was improvement in the neurological assessment from 0 to 7 months (Table 4.5). Hard et al. (2000) showed that in preterm children, who are often deprived of AA and DHA *in utero*, visual perceptual problems where prevalent compared with control subjects when assessed at 5.1-9.3 years of age by TVPS-R assessment. Early nutrition intervention is important, as this is a critical time and can have long lasting consequences on visual perception later in childhood. In the present study, when DHA intake (mg/day) was compared with median visual perceptual age (months), the control and LCP groups are distributed differently. Level of DHA intake had an apparent effect on the median visual perceptual age derived (Figure 4.3).

Visual discrimination (one of the subsets of TVPS-R) is similar to the Vernier acuity, which is defined as the ability to detect small changes in alignment or position (Neuringer and Jeffrey 2003). In infancy, behavioural methods (such as forced preferential looking which requires an infant to discriminate between two patterns) or visual evoked potentials are used to assess Vernier acuity as reviewed by Neuringer and Jeffrey (2003). These two methods are used in a preverbal child and are an indirect measure of vision as tested by letter recognition. Visual spatial-relationships (one of the subsets of TVPS-R) is similar to grating acuity, which is defined as spatial threshold for

resolving dark and light stripes (Neuringer and Jeffrey 2003). Grating acuity is assessed in infancy using forced preferential looking (preference of the infant for looking at a high-contrast patterns to determine the smallest stripes that evoke a detectable response) and sweep visual evoked potential similar to those used to assess Vernier acuity (Neuringer and Jeffrey 2003).

4.4.4 Individual Fatty Acids (AA and DHA in plasma phospholipids and RBC phospholipids)

AA and DHA levels in the RBC phospholipid and the plasma was plotted for all subjects showed a general trend toward a higher level of AA and DHA in the LCPUFA group compared to the control group at 7 months, however this was not significantly different between treatment groups when assessed using ANOVA (Figure 4.4 and Figure 4.5). AA and DHA is incorporated to where it is needed in the body, therefore an increased level of AA and DHA may not be present in the plasma until all the body needs of AA and DHA are satisfied. When assessing fatty acid status in blood lipids, it is important to quantitatively assess the absolute value of AA and DHA content in the plasma phospholipids (ug/mL) and not just the relative % wt/wt of AA and DHA in the RBC.

Clandinin et al. (1995), showed that in pediatric subjects, who were located geographically in similar area to subjects in the present study, had plasma phospholipid DHA values (5.9± 4.0 ug/mL), lower than many control subjects in the present study. Supplementation with fish oil (35 mg n-3 fatty acids/ kg body weight for 4 weeks) increased the plasma DHA values by approximately 2.5 fold, indicating that the level of

DHA supplemented was effective in increasing plasma DHA levels (Clandinin et al. 1995).

Clandinin et al. (1997) supplemented preterm infants with one of four formulas containing increasing levels of AA and DHA ranging from 0 to 1.1% AA and 0 to 0.75% DHA, while another group was provided with human milk. Providing formula containing increasing levels of AA and DHA resulted in a clear dose response in the AA and DHA in plasma phospholipids (Clandinin et al. 1997). Plasma levels of AA and DHA declined in infants not fed AA and DHA indicating that infants have limited ability synthesize AA and DHA from its dietary precursors (Clandinin et al. 1997). In the present study, children supplemented with LCP, had AA and DHA levels similar to plasma phospholipids AA and DHA levels in preterm infants fed LCP formula containing 0.32% AA and 0.24% DHA or human breast milk (0.54% AA and 0.30% DHA) (Clandinin et al. 1997).

4.5 Conclusion

There is limited research investigating vision and brain development beyond 2 years of age even though neurological growth continues throughout childhood. The present study is the first to describe the effect of providing a nutritionally complete dietary supplement containing AA and DHA to healthy children 5-7 years of age, who were previously identified to consume low levels of DHA (Chapter 3). Supplementing children with AA and DHA for 7 months improved visual perception. Furthermore, children that were supplemented with LCP, had AA and DHA levels similar to plasma phospholipids AA and DHA levels in preterm infants fed LCP formula containing 0.32%

AA and 0.24% DHA or human breast milk (0.54% AA and 0.30% DHA). These results suggest that children should consume specific foods that contain AA and DHA as the level of both these fatty acids may have a positive impact on visual perception.

4.6 References

Agostoni C, Trojan S, Bellu R, Riva E, Bruzzese MG, Giovannini M (1997) Developmental quotient at 24 months and fatty acid composition of diet in early infancy: a follow up study. Archives of Disease in Childhood 76:421-424

Agostoni C, Trojan S, Bellu R, Riva E, Giovannini M (1995) Neurodevelopmental quotient of healthy term infants at 4 months and feeding practice: the role of long-chain polyunsaturated fatty acids. Pediatric Research 38:262-266

Allison DB, Egan K, Barraj LM, Caughman C, Infante M, Heimbach JT (1999) Estimated intakes of trans fatty and other fatty acids in the US population. Journal of the American Dietetic Association 99:166-174

Bausell RB, Li Y-F (2002) Power analysis for experimental research: a practical guide for the biological, medical and social sciences. Cambridge University Press, Cambridge, UK

Birch EE, Birch DG, Hoffman DR, Uauy R (1992) Dietary essential fatty acid supply and visual acuity development. Investigative Ophthalmology and Visual Science 33:3242-3253

Birch EE, Hoffman DR, Castaneda YS, Fawcett SL, Birch DG, Uauy RD (2002) A randomized controlled trial of long-chain polyunsaturated fatty acid supplementation of formula in term infants after weaning at 6 wk of age. American Journal of Clinical Nutrition 75:570-80

Birch EE, Hoffman DR, Uauy R, Birch DG, Prestidge C (1998) Visual acuity and the essentiality of docosahexaenoic acid and arachidonic acid in the diet of term infants. Pediatric Research 44:201-209

Briefel R, Flegal K, Winn D, Loria C, Johnson C, Sempos CT (1992) Assessing the nation's diet: limitations of the food frequency questionnaire. Journal of the American Dietetic Association 92:959-962

Burdge G, Finnegan YE, Minihane AM, Williams CM, Wootton SA (2003) Effect of altered n-3 fatty acid intake upon plasma lipid fatty acid composition, conversion of [13C]alpha-linolenic acid to longer-chain fatty acids and partitioning towards b-oxidation in older men. British Journal of Nutrition 90:311-321

Burdge G, Jones AE, Wootton SA (2002) Eicosapentaenoic and docosapentaenoic acids are the principal products of alpha-linolenic acid metabolism in young men. British Journal of Nutrition 88:355-363

Carlson SE, Werkman SH (1996) A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until two months. Lipids 31:85-90

Carlson SE, Werkman SH, Rhodes P, Tolley E (1993) Visual acuity development in healthy preterm infants: effect of marine-oil supplementation. American Journal of Clinical Nutrition 58:35-42

Carnielli VP, Wattimena DJ, Luijendijk IH, Boerlage A, Degenhart HJ, Sauer PJ (1996) The very low birth weight premature infant is capable of synthesizing arachidonic and docosahexaenoic acids from linoleic and linolenic acids. Pediatric Research 40:169-174

Chugani HT (1998) A critical period of brain development: studies of cerebral glucose utilization with PET. Preventive Medicine 27:184-188

Clandinin M, Chappell J, Heim T (1982) Do low weight infants require nutrition with chain elongation-desaturation products of essential fatty acids? Progress in Lipid Research 20:901-904

Clandinin M, Chappell J, Heim T, Swyer P, Chance G (1981) Fatty acid utililzation in perinatal de novo synthesis. Early Human Development 5:355-366

Clandinin M, Chappell J, Leong S, Heim T, Swyer P, Chance G (1980) Intraunterine fatty acid accretion rates in human brain: implications for fatty acid requirements. Early Human Development 4:121-129

Clandinin M, Van Aerde J, Parrott A, Field C, Euler A, Lien E (1997) Assessment of the efficacious dose of arachidonic and docosahexaenoic acids in preterm infant formulas: fatty acid composition of erythrocyte membrane lipids. Pediatric Research 42:819-825

Clandinin MT, Van Aerde JE, Merkel KL, Harris CL, Springer MA, Hansen JW, Diersen-Schade D (2005) Growth and development of preterm infants fed infant formulas containing docosahexaenoic acid and arachidonic acid. The Journal of Pediatrics

Clandinin MT, Zuberbuhler P, Brown NE, Kielo ES, Goh YK (1995) Fatty acid pool size in plasma lipoprotein fractions of cystic fibrosis patients. American Journal of Clinical Nutrition 62:1268-1275

Dobbing J (1972) Vulnerable periods in of brain development. In: K E, Knight J (eds) Lipids, Malnutrition and the developing brain. Elsevier, Amsterdam, pp 9-20

Emken E, Adlof R, Gulley RM (1994) Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. Biochimica et Biophysica Acta 1213:277-288

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipides from animal tissues. The Journal of Biological Chemistry 226:497-509

Gardner MF (1996) TVPS-R Test of Visual-Perceptual Skills (non-motor)-Revised Manual. Psychological and Educational Publications, Inc., Hydesville, CA

Garey L (1984) Structural development of the visual system of man. Human Neurobiology 3:75-80

Garey LJ (1983) Development of visual system- comparison of monkey and man. Acta Morphologica Academiae Scientiarum Hungaricae 31:27-38

Gibson R (1990) Principles of Nutritional Assessment. Oxford University Press, New York

Hard A-L, Niklasson A, Svensson E, Hellstrom A (2000) Visual function in school-aged children born before 29 weeks of gestation: a population-based study. Developmental Medicine and Child Neurology 42:100-105

Hedin A, Nymann K, Derouet B (1980) A modified letter matching chart for testing young children's visual acuity. Journal of Pediatric Ophthalmology and Strabismus 17:114-118

Hoffman DR, Birch EE, Castaneda YS, Fawcett SL, Wheaton DH, Birch DG, Uauy R (2003) Visual function in breast-fed term infants weaned to formula with or

without long-chain polyunsaturates at 4 to 6 months: a randomized clinical trial. Journal of Pediatrics 142:669-677

Hussein N, Ahmad A, Wilkinson P, Leach C, Griffin BA, Millward DJ (2005) Long-chain conversion of [13C]linoleic acid and alpha-linolenic acid in response to marked changes in their dietary intake in men. Journal of Lipid Research 46:269-280

Huttenlocher PR, de Courten C, Garey LJ, Van Der Loos H (1982) Synaptogenesis in human visual cortex- evidence for synapse elimination during normal development. Neuroscience Letters 33:247-252

Innis SM (2003) Perinatal biochemistry and physiology of long-chain polyunsaturated fatty acids. Journal of Pediatrics 143:S1-S8

Innis SM, Vaghri Z, King DJ (2004) n-6 Docosapentaenoic acid is not a predictor of low docosahexaenoic acid status in Canadian preschool children. American Journal of Clinical Nutrition 80:768-73

Institute of Medicine (1997) Dietary reference intakes for calcium, phosphorus, magnesium, vitamin D, and fluoride. National Academy Press, Washington, DC

Institute of Medicine (1998) Dietary reference intakes for thiamin, riboflavin, niacin, vitamin B6, folate, vitamin B12, pantothenic acid, biotin and choline. National Academy Press, Washington, DC

Institute of Medicine (2000) Dietary Reference Intakes for vitamin C, vitamin E, selenium and carotenoids. National Academy Press, Washington, DC

Institute of Medicine (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. National Academy Press. Washington, DC

Institute of Medicine (2002) Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids (macronutrients). National Academies Press. Washington, DC

Institute of Medicine (2004) Dietary reference intakes for water, potassium, sodium, chloride, and sulfate. National Academies Press. Washington, DC

Jonnalagadda SS, Egan KS, Heimbach JT, Harris SS, PM K-E (1995) Fatty acid consumption pattern of Americans: 1987-1988 USDA Nationwide Food consumption survey. Nutrition Research 15:1767-1781

Lakowski R (1965) Colorimetric and photometric data for the 10th edition of the Ishihara plates. British Journal of Physiological Optics 22:195-207

Lang J (1983) A new stereotest. Journal of Pediatric Ophthalmology and Strabismus 20:72-74

Levitt P (2003) Structural and functional maturation of the developing primate brain. Journal of Pediatrics 143:S35-45

Lien V, Clandinin MT (unpublished) Assessment of docosahexaneoic acid, arachidonic acid, vitamin A, vitamin E, and iron nutrient intakes in 4-7 year old children.

Meyer BJ, Mann NJ, Lewis JL, Milligan GC, Sinclair A, Howe PR (2003) Dietary intakes and food sources of omega 6 and omega 3 polyunsaturated fatty acids. Lipids 38:391-398

Morrison WR, Smith LM (1964) Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research 5:600-608

Neuringer M, Jeffrey BG (2003) Visual development: neural basis and new assessment methods. J Pediatr 143:S87-S95

Oyster CW (1999) The Human Eye- Structure and Function. Sinauer Associates Inc, Sunderland, Massachusetts

Paus T, Collins D, Evans A, Leonard G, Pike B, Zijdenbos A (2001) Maturation of white matter in human brain: a review of magnetic resonance studies. Brain Research Bulletin 54:255-266

Rose HG, Oklander M (1965) Improved procedure for the extraction of lipids from human erythrocytes. Journal of Lipid Research 6:428-431

Salem NJ, Pawlosky RJ, Wegher B, Hibbeln JR (1999) In vivo conversion of linoleic acid to arachidonic acid in human adults. Prostaglandins, Leukotrienes and Essential Fatty Acids 60:407-410

Salem NJ, Wegher B, Mena P, Uauy R (1996) Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. Proceedings of the National Academy of Sciences of the United States of America 93:49-54

Sastry P (1985) Lipids of nervous tissue: composition and metabolism. Progress in Lipid Research 24:66-176

Sauerwald T, Hachey D, Jensen C, Chen H, Anderson RE, Heird W (1996) Effect of dietary alpha-linolenic acid intake on incorporation of docosahexaenoic and arachidonic acids into plasma phospholipids of term infants. Lipids 31:S131-S135

Sempos CT (1992) Invited commentary: some limitations of semiquantitative food frequency questionnaires. American Journal of Epidemiology 135:1127-1132

Szitanyi P, Koletzko B, Mydlilova A, Demmelmair H (1999) Metabolism of 13C-Labeled Linoleic acid in newborn infants during the first week of life. Pediatric Research 45:669-673

Tinoco J (1982) Dietary requirements and functions of a-linolenic acid in animals. Progress in Lipid Research 21:1-45

U.S. Department of Agriculture ARS (1998) USDA Nutrient Database for Standard Reference, Release 12. Nutrient Data Laboratory Home Page, http://www.nal.usda.gov/fnic/foodcomp.

Werkman SH, Carlson SE (1996) A Randomized Trial of Visual Attention of Preterm Infants Fed Docosahexaenoic Acid Until Nine Months. Lipids 31:91-97

Williams C, Birch EE, Emmett PM, Northstone K, Avon Longitudinal Study of Pregnancy and Childhood (ALSPAC) Study Team (2001) Stereoacuity at age 3.5 y in children born full-term is associated with prenatal and postnatal dietary factors: a report from a population-based cohort study. American Journal of Clinical Nutrition 73:316-322

Chapter 5- Final Summary Discussion and Conclusions

5.1 General Summary

Dietary intake of AA, DHA, vitamin A, vitamin E, and iron by children living in central Alberta, was assessed using a 3-day food record (**Objective 1**). The dietary intakes of AA and DHA assessed in Objective 1 have been expressed in relation to body weight (mg/kg) and compared with AA and DHA intakes (mg/kg) of infants fed human breast milk and infant formulas containing AA and DHA (**Objective 2**). Children, who have been identified to consume low intakes of DHA, were supplemented with AA and DHA to determine if the supplement will increase essential fatty acid status and improve visual perception in children for a period of 7 months (**Objective 3**).

Hypothesis 1

It was hypothesized that children (4-7 years of age) living in central Alberta will consume diets low in DHA. Results obtained from 3-day food records confirm that children studied in central Alberta consume diets that are low in DHA (74% of subjects had DHA intakes of $\leq$30 mg/day) (Chapter 3).

Hypothesis 2

It was hypothesized that supplementing children who have been identified to consume low intakes of DHA, with a nutritionally complete dietary supplement containing with AA and DHA, will lead to an increase in individual fatty acids (AA and DHA in plasma and red blood cell phospholipids), and an improvement of visual perception compared to

children who are not supplemented with AA and DHA for a period of 7 months. Results showed that daily supplementation of AA and DHA in children for a period of 7 months increased AA phospholipids in the plasma over 7 months within the LCPUFA group, whereas DHA phospholipids in the RBC decreased over 7 months within the control group (Chapter 4). Supplementation of healthy 5-7 year old children, who were previously identified to consume low dietary intakes of DHA, also showed an improvement in visual perception compared to children that were not supplemented with AA and DHA (Chapter 4).

5.2 Conclusions

The main contributions of this thesis are as follows:

1. Children, 4-7 years of age, living in central Alberta consume diets that are low in DHA.
2. Children living in central Alberta consumed lower levels of AA (2.28 mg/kg) and DHA (1.48 mg/kg) compared to infants fed human breast milk (AA 33-178 mg/kg; DHA 18-99 mg/kg) or infants fed formula containing AA (154-185 mg/kg) and DHA (77.2-92.6 mg/kg)
3. Healthy 5-7 year old children, who were previously identified to consume low dietary intakes of DHA, when provided with a nutritionally complete dietary supplement containing AA (20-30 mg/day) and DHA (14-21 mg/day) showed an improvement in visual perception.

4. Providing a nutritionally complete dietary formula (2-3 sachets/per day) lead to a decreased food consumption of DHA containing foods in all subjects over 7 months.
5. Providing a nutritionally complete dietary formula (2-3 sachets/per day) lead to a significant increase in AA containing foods in all subjects over 7 months.
6. Providing a nutritionally complete dietary formula containing AA and DHA, showed a general trend toward a higher level of AA and DHA in the RBC phospholipid and the plasma phospholipids within the LCPUFA group compared to the control group at 7 months.
7. Providing a nutritionally complete dietary supplement to both the Control and LCPUFA group did not increase overall energy intake of the children during the 7 month study when assessed on a per body weight basis.

5.3 Overall Conclusion, Limitations and Future Research

5.3.1 Study 1

The first study (Chapter 3), investigated the nutrient intakes of AA, DHA, Vitamin A, Vitamin E, and Iron in children 4-7 years of age. The significance of the results from the first study is that children, who geographically do not live near a marine environment, have low intake of AA and DHA when assessed using a 3-day food record. Another contribution from the first study is that AA and DHA intakes were expressed in relation to body weight (mg/kg) and compared with infants fed human breast milk and infant formulas containing AA and DHA. In the first study, children had considerably lower AA and DHA intakes than in infants fed human breast milk and infant formulas

containing AA and DHA. Since brain and eye neural development continues throughout childhood, low intake of AA and DHA may have a negative impact on retinal and neuronal development. Future research is necessary to investigate if supplementing children with low intakes of DHA can improve vision and brain development, which leads to the research conducted in the second study.

A limitation of the first study is use of a 3-day unweighed food record. Use of a 7-day food record maybe considered a more precise method of estimating usual food and/or nutrient intakes of individuals, so long as respondents do not change their usual eating pattern (Gibson 1990). This method was considered to be too much of a burden to parents participating in the present study and would have resulted in problems with compliance and completion rates.

5.3.2 Study 2

In the second study (Chapter 4) children, who were previously identified to consume low intakes of DHA (Chapter 3), were provided with a nutritionally complete dietary supplementing containing AA (20-30 mg/day) and DHA (14-21 mg/day) and essential fatty acids status and visual perception was evaluated. The significance of the results in the second study is that providing children, who previously consumed low intake of DHA, showed an improvement in visual perception, and a general trend towards a higher level of AA and DHA in the RBC phospholipid and the plasma phospholipids within the LCPUFA group compared to the control group at 7 months.

Providing a nutritionally complete dietary supplement to both the control and LCPUFA group did not increase overall energy intake of the children during the 7 month study when assessed on a per body weight basis. Majority of the children reduced their

food intake while consuming the dietary supplement to compensate for the additional calories provided by the dietary supplement, therefore taking the dietary supplement will not likely cause unhealthy weight gain in children since the supplement did not increase overall energy intakes.

There are some limitations of the second study including the dose of AA and DHA supplemented to the children, and the length of time children were supplemented. Providing a dose of 20-30 mg/day of AA and 14-21 mg/day DHA, showed a consistent trend of higher individual fatty acids (AA and DHA in plasma and red blood cell phospholipids), however, the results were not significant between the LCPUFA and the control group. Children who had DHA intakes of less than 78 mg/day in the first study were recruited in the second study, of which 34 of 37 children had DHA intakes less than 30 mg/day. Therefore providing the children with a supplement that contained AA and DHA, would increase the current DHA intake level by at least 50% in the majority of the children. An unexpected outcome of the second study, which has complicated findings, is that all subjects decreased intake of DHA containing foods (50% decrease in control group and 35% decrease in the LCPUFA group). Supplementing a higher dose of AA and DHA might also compensate for any decrease in the consumption of DHA containing foods and may further increase individual fatty acid levels (AA and DHA in plasma and red blood cell phospholipids) in the LCPUFA group compared to the Control group. For instance, a dose calculated per body weight basis, such as 35 mg/day n-3 which have been used in another study (Clandinin et al. 1995) or providing a dose of AA and DHA provided to infants fed human breast milk (AA 33-178 mg/day; DHA 18-99 mg/kg) are reasonable target doses for future studies.

In the second study, children were provided with a nutritionally complete milk-based dietary supplement with AA and DHA (LCPUFA group) and without AA and DHA (control group). The formula was packaged in a sachet (dry powder form), where parents were required to reconstitute the formula with the addition of water. Although it would have been beneficial to supplement a higher dose of AA and DHA to the child, there are few products in the market that are available. The purpose of providing a supplement in a beverage was to deliver AA and DHA in a nutritionally balanced food format (not a pill format), that children would enjoy, that may be useful in school-based lunch programs.

The length of time the children spent in the study is another possible limitation. In the present study, children 5-7 years of age were supplemented with LCPUFA for a period of 7 months. Eye and brain development continue in childhood (Chugani 1998; Dobbing 1972; Oyster 1999), and synaptogenesis (a period of growth spurts in the brain), peaks at eight months and reaches adult values by 11 years (Garey 1984; Garey 1983; Huttenlocher et al. 1982). Therefore, supplementing children for up to 11 years of age may result in further benefits in neurological function and is a reasonable target for future studies.

In the second study, both treatment groups (control and LCP) were required to consume a nutritionally complete dietary supplement. It would have been beneficial to have a third group that did not undergo any dietary intervention. This would provide a greater understanding to why intake of DHA containing foods decreased over time (does this naturally occur in the diet of children over time or was it the result of taking a dietary supplement). More research is needed to determine if supplementing children with a

higher dose of AA and DHA (calculated on a per body weight basis) or a longer duration, results in further benefits in neurological function.

5.4 References

Chugani HT (1998) A critical period of brain development: studies of cerebral glucose utilization with PET. Preventive Medicine 27:184-188

Clandinin MT, Zuberbuhler P, Brown NE, Kielo ES, Goh YK (1995) Fatty acid pool size in plasma lipoprotein fractions of cystic fibrosis patients. American Journal of Clinical Nutrition 62:1268-1275

Dobbing J (1972) Vulnerable periods in of brain development. In: K E, Knight J (eds) Lipids, Malnutrition and the developing brain. Elsevier, Amsterdam, pp 9-20

Garey L (1984) Structural development of the visual system of man. Human Neurobiology 3:75-80

Garey LJ (1983) Development of visual system- comparison of monkey and man. Acta Morphologica Academiae Scientiarum Hungaricae 31:27-38

Gibson R (1990) Principles of Nutritional Assessment. Oxford University Press, New York

Huttenlocher PR, de Courten C, Garey LJ, Van Der Loos H (1982) Synaptogenesis in human visual cortex- evidence for synapse elimination during normal development. Neuroscience Letters 33:247-252

Oyster CW (1999) The Human Eye- Structure and Function. Sinauer Associates Inc, Sunderland, Massachusetts

Appendix A

Table A.1 Fatty Acid Profile Content of Control and LCP Supplement Per Sachet Serving[1]

Fatty acid	Control[2] Per sachet (1 serving) mg per 200 mL	LCP[3] Per sachet (1 serving) mg per 200 mL
Saturated		
Caprylic C8:0	129.6	84.0
Capric C10:0	122.4	72.8
Lauric C12:0	720	521
Myristic C14:0	324	241
Palmitic C16:0	1584	1142
Stearic C18:0	324	246
Arachidic C20:0	21.6	16.8
Behenic C22:0	14.4	11.2
Lignoceric C24:0	-	5.60
Total Saturated	3240	2340
Monounsaturated		
Palmitoleic C16:1	7.20	11.2
Oleic C18:1	2664	1999.2
Eicosanic C20:1	14.4	11.2
Total monounsaturated	2685.6	2021.6
Polyunsaturated		
Linoleic C18:2n-6	1166.4	1086.4
Linolenic C18:3n-3	108	123.2
Arachidonic C20:4n-6	-	10.4
Docosahexaenoic C22:6n-3	-	7.00
Total polyunsaturated	1274.4	1227

[1]One sachet serving = 200 mL
[2]Control supplement (no LCP)
[3]LCP supplement (with LCP)

Appendix B

Table B.1 Nutrient Composition of Control and LCP Supplement Fed

Nutrients	Per 1 sachet (200 mL serving)
Energy (kcal)	134
Protein (g)	4.40
Carbohydrate (g)	16.4
80% lactose (g)	13.1
20% sucrose (g)	3.28
Fat (g)	5.60
Vitamin A (IU)	500
Vitamin D (IU)	85.0
Vitamin E (IU)	2.20
Vitamin K (mcg)	13.4
Vitamin B1 (mcg)	200
Vitamin B2 mcg)	300
Vitamin B6 (mcg)	120
Vitamin B12 (mcg)	0.400
Niacin (mg)	0.800
Folic Acid (mcg)	16.0
Pantothenic acid (mcg)	600
Biotin (mcg)	4.00
Vitamin C (mg)	18.0
Choline (mg)	20.0
Calcium (mg)	230
Phosphorus (mg)	130
Magnesium (mg)	17.0
Iron (mg)	2.40
Zinc (mg)	1.00
Manganese (mcg)	20.0
Copper (mcg)	80.0
Iodine (mcg)	26.0
Sodium (mg)	70.0
Potassium (mg)	200
Chloride (mg)	150
Selenium (mcg)	2.80

Appendix C

Food Frequency Questionnaire

Estimate your usual consumption of the following foods. Use the food model forms to help estimate portion size.

1. How many 3oz servings of the following fish does your child eat **monthly**?

a) bluefish: _____
b) bluefin tuna:_____
c) cisco, smoked:_____
d) herring:_____
e) mackerel:_____
f) Pollock:_____
g) sardines:_____
h) salmon:_____
i) whitefish:_____

2. How many 3 oz servings of the following fish/shellfish does your child eat **monthly**?

a) bass:____
b) catfish:_____
c) drumfish:_____
d) flounder:_____
e) grouper:_____
f) mussels:_____
g) perch:_____
h) redfish:_____
i) rockfish:_____
j) shark:_____
k) snapper:_____
l) sole:_____
m) swordfish:_____
n) trout:_____
o) tuna, canned (1 cup):_____

3. How many 3 oz servings of the following fish/shellfish does your child eat **monthly**?

a) carp:_____
b) clams:_____
c) cod:_____
d) crab:_____
e) crayfish:_____
f) fish sticks:_____
g) fish patties/ squares:_____
h) haddock:_____
i) lobster:_____
j) mullet:_____
k) oysters:_____
l) pampano:_____
m) pike:_____
n) scallops:_____
o) shrimp:_____
p) sturgeon:_____

4. List other fish/shellfish consumed **not** on the above list (list amount the number of 3 oz servings of the fish/shellfish consumed **monthly**)

Name of fish/shellfish:_____
Number of 3 oz. Servings of fish/shellfish consumed **monthly**:
_____

5. How many 3 oz servings of liver (chicken, turkey, beef) does your child eat **monthly**?
_____

6. How many egg yolks does your child eat **weekly** (including eggs yolks used in cooking)? _____

7. What type of eggs do you consume (e.g. omega-3 enriched eggs, regular eggs from supermarket, farm bought eggs)? _____

8. How many 3 oz servings of chicken, turkey or other poultry (not including livers) does your child eat **weekly?** _____

Appendix D

Figure D.1 Sample HOVT chart Used to Assess Visual Acuity in Children (Hedin et al. 1980)

Caption: Visual acuity (the minimum discrimination of an object at a fixed distance) was determined using the HOVT chart) (Hedin et al. 1980). This chart consists of four random letters (HOVT) of different sizes, and was placed 10 feet away, while the child was required to read off the letters while one eye was covered. Left and right eyes were tested separately, and the final score of correct letters read out of 44 was recorded.

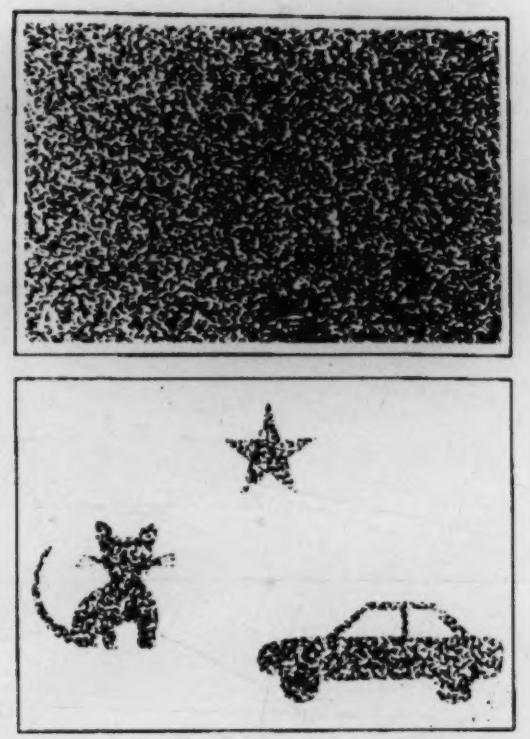


Figure D.2 Sample Lang Test Used to Assess Stereo Acuity in Children (Lang 1983)

Caption: The Lang test was used to measure stereo acuity (depth perception) (Lang 1983). This test consists of random dots, where an image can be found within the random dots and can only be seen if the child has stereo viewing. A yes or no response was recorded.

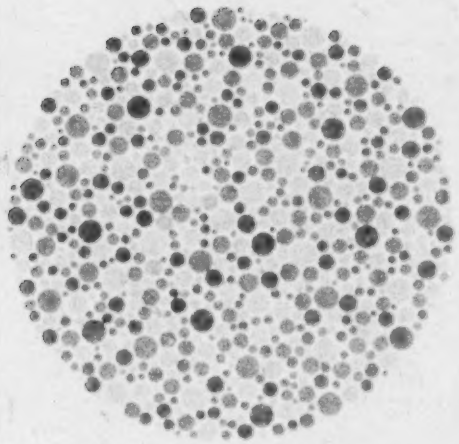


Figure D.3 Sample Ishihara Pseudoisochromatic Plate Used to Assess Colour Vision in Children (Lakowski 1965)

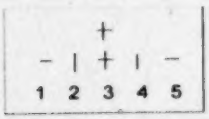
Caption: Colour vision (the ability of the child to distinguish between different colours) was assessed using Ishihara pseudoisochromatic plates (Lakowski 1965). The plates have multicolour dots which form numbers when the same hue and saturation of colour is present. Individuals who are colour blind are unable to detect certain numbers or may see different numbers than subjects who are not colour blind. A modified test was used where only eight plates (with single digit numbers) were shown to the child, instead of using plates that had two digits. Left and right eyes were tested and a score out of 8 was recorded for each eye.

Figure D.4 Prism Cover Test was Used to Assess Strabismus

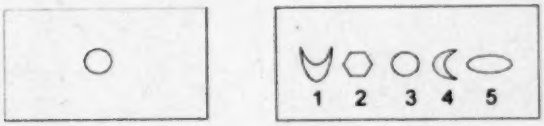
Caption: The prism cover test was used to determine if a child had strabismus (cross-eyed). One eye would be covered with a paddle, and if the uncovered eye shifted or moved, then a prism was used to determine the angle of movement in the eye. This was recorded at both near (reading distance) and at a distance (20 feet or 6 metres).

Appendix E

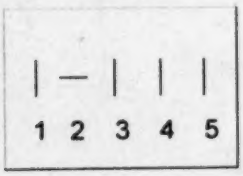
Visual Discrimination



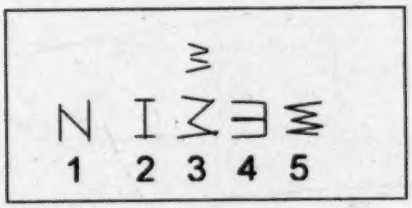
Visual Memory



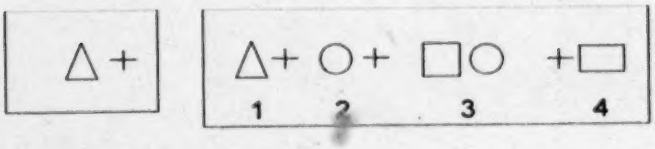
Visual Spatial-Relationships



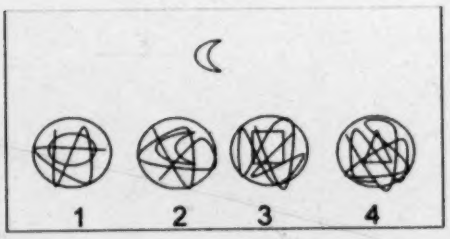
Visual Form-Constancy



Visual Sequential-Memory



Visual Figure- Ground



Visual Closure

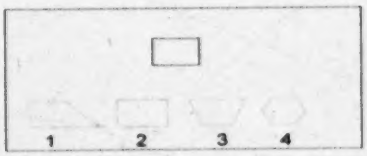


Figure E.1 Sample Graphical Representation of Each of the 7 Subsets in the Test of Visual Perceptual Skills (non-motor)-Revised (TVPS-R) (Gardner 1996).

Caption: The Test of Visual Perceptual Skills (non-motor)-Revised (TVPS-R) is a standardized test developed for children 4 years through 12 years, and measures visual perceptual skills using seven subtests: visual discrimination (identification and matching of similar forms); visual memory (immediate recall of a single form); visual spatial-relationships (identification of the correct direction of forms); visual form-constancy (identification of a form, whether it is a different size, rotated, reversed, or hidden in or among other forms); visual sequential-memory (immediate recall of a number of forms in a series); visual figure-ground (finding a form that is hidden among other forms); visual closure (determination of a whole form from incomplete parts of the form).

E. References

Gardner MF (1996) TVPS-R Test of Visual-Perceptual Skills (non-motor)-Revised Manual. Psychological and Educational Publications, Inc., Hydesville, CA

Hedin A, Nymann K, Derouet B (1980) A modified letter matching chart for testing young children's visual acuity. Journal of Pediatric Ophthalmology and Strabismus 17:114-118

Lakowski R (1965) Colorimetric and photometric data for the 10th edition of the Ishihara plates. British Journal of Physiological Optics 22:195-207

Lang J (1983) A new stereotest. Journal of Pediatric Ophthalmology and Strabismus 20:72-74